



# Cereal Straw as a Resource for Sustainable Biomaterials and Biofuels

Chemistry, Extractives, Lignins, Hemicelluloses and Cellulose



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The use of materials from renewable resources is attaining increased importance, and the world's leading industries and manufacturers are seeking to replace dwindling petrochemical-based feedstocks with products derived from natural biomass such as cereal straws, which consist mainly of three groups of organic compounds—cellulose, hemicelluloses, and lignin—representing an abundant, inexpensive, and readily available source of renewable lignocellulosic biomass. In particular, the whole concept of the chemical aspect of straw utilization was brought sharply into focus late in the 1990s when the world was faced with the issue of oil and material shortage. On the basis of 30 years of work in this area, I am convinced that cereal straw will play an important role in moving society from its petrochemical dependency to a sustainable economy, and I also note that to use straw wisely and judiciously, we need to have a basic knowledge of its composition and the structure of the cell walls. Chemical studies of straw and its components may provide decisive factors not only for its applicability but also for the economic feasibility of many industrial processes involving straw. However, although several excellent books on wood chemistry were published in the past, there is no book devoted specifically to straw chemistry. More importantly, only recently new processes have been developed where straw components are separated and isolated on the pilot scale. All of these reasons provided the inspiration for this book.

This book provides us a thorough overview of straw chemistry. [Chapter 1](#) provides an introduction. [Chapter 2](#) is concerned with the fundamentals of straw structure, ultrastructure, and chemical composition. [Chapter 3](#) covers the extractives, which include isolation and structural characterization. [Chapters 4–6](#) review what is currently

known about the three main components with respect to their discovery, occurrence, chemical and physical properties, analysis, molecular weight, isolation, and application. The last chapter focuses on chemical modifications of straw as novel materials for various industries.

This book summarizes both our knowledge of straw chemistry and gives a comprehensive account of progress and current knowledge in straw chemistry, drawing on an extensive set of references. I sincerely hope that this book will be used as a teaching book and anticipate that it will be particularly useful not only to scientists with research interests in the areas of natural resource management, environmental chemistry, plant chemistry, material science, polysaccharide chemistry, lignin chemistry, etc., but also to academic and industrial scientists/researchers with an interest in using agricultural residues as novel products for industries and/or recycling technologies. I am sure that this book will play an important role in the process of utilization of straw.

All of the contributors, who were carefully selected to cover each particular subject, are active in research. As a result, this book provides, not only a comprehensive picture of the state of the art, but also much unpublished data from recent studies. I sincerely thank all the contributing authors, who come from Beijing Forestry University, South China University of Technology, University of Wisconsin-Madison, and North-West Agriculture and Forestry University, for their excellent collaboration. I also would like to give special thanks to Fachuang Lu and John Ralph for their long-term cooperation; [Chapter 6](#) contains some unpublished data from their recent studies.

*Run-Cang Sun  
Beijing, 2009*



# Introduction

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Straw, the above-ground part of the cereal plant that remains after the nutrient grain or seed has been removed, comprises about half the total dry weight of the crop. For many centuries, straw was valued as the most useful by-product of cereal production, and it has been used for feeding livestock, bedding, growing mushroom, and so on [1]. However, with the development of science and technology in the recent decades, especially with the exploitation of petroleum, straw is regarded as little more than an embarrassing companion to the grain crop. Farmers in many of the chief cereal-growing countries of the world burn or plough the straw into the field directly as a fertilizer. For instance, in the United Kingdom, approximately half (6 million tons) of the annual production of recoverable cereal straw in England and Wales are disposed of by burning [2]. It was reported that only a little of the straw remaining from the nearly 230 million hectares of wheat grown annually in the world was used as a fodder for animals [3].

However, as petroleum is currently one of the most important natural resources and a raw material for the synthesis of various chemicals, a series of issues has arisen, such as the diminishing world reserves of petroleum, the resultant pollution from the processing and utilization of petroleum, the sensitive petroleum market, and so on. Especially, the problem of global warming requires severe reductions in the use of fossil fuel [4]. In addition, the straw burning should be avoided, as it causes serious environmental pollution. These problems have led researchers to pay attention to the value of biomass, which is both sustainable and CO<sub>2</sub>-neutral. Agricultural crop residues, such as straws of wheat, barley, rice, maize, oats, rye, and cotton, as well as sugarcane bagasse and other residues, represent an enormous underutilized energy resource, which has a great potential as feed for ruminants and also as raw materials for paper, chemicals, and other technical products [5]. Generally, for every ton of cereal production worldwide, about 1.5 tons of straw is obtained as a by-product. World production of cereals exceeds 1000 million tons per

annum, which means about 1500 million tons of cereal straw is produced each year, in which china produce more than 700 million tons cereal straws per year [6]. Straw and other fibrous by-products from cereals available in the world amount to approximately 3000 million tons per year [7]. Because of the enormous quantity of straw, utilization of straw to the utmost extent is now demanding attention in the major cereal-growing areas of the world.

One of the most traditional utilizations of straw is as feed for livestock. Unfortunately, we notice that even though straw contains enough cellulose, which makes it an excellent source of energy for ruminants, it is a poor-quality feed in its natural state. The limited use of straw as feed is due to its low rate of degradation in the rumen, low digestibility, and low voluntary intake [8, 9]. This is caused by the chemical structure of the straw, which limits the digestion of cellulose and hemicelluloses. The chemical factors include lignification [10, 11], silicification, crystallinity of cellulose, and other factors [12]. The problem of straw being used for livestock feeding is discussed in detail by Han et al. [12] and Morrison [13].

Various physical, chemical, and biological treatments have been applied to improve utilization of straws. Physical treatment is carried out mainly to increase the surface area, which would enhance the attachment of bacteria. Processes such as milling, grinding, chopping, and steaming have long been used to improve the feed value (digestibility) of straw [14]. Chemical methods have been used to improve the digestibility of wheat straw [15, 16]. Alkaline treatment of lignocellulosic substances such as wheat straw disrupts the cell wall by dissolving hemicelluloses, lignin, and silica, by hydrolyzing uronic and acetic acid esters and by swelling cellulose, and by decreasing the crystallinity of cellulose [9]. This increase of the biodegradability of the cell walls is also due to the cleavage of the bonds between lignin and hemicelluloses or lignin and phenolic acids. In general, ammonium hydroxide (NH<sub>4</sub>OH) usually produces a positive response [17–20] but is generally less effective than

NaOH [9, 21]. Advantages of  $\text{NH}_4\text{OH}$  over the mineral hydroxides are excess of evaporation, elimination of mineral imbalances, and supply of supplemental N [9, 22]. The most promising results have been obtained from biological methods of degrading lignin. The use of intact microorganisms or their enzymes for the conversion of straw into animal feed has been an active area of research [23–26]. One of the major ligninolytic organisms, the white rot fungus, has been used extensively in this area of research. Although a huge potential is found in the utilization of straw as an animal feed, we need to consider the ways in which the carbohydrates and lignin in straw are degraded and how these components interact with each other to prevent degradation.

The demand for paper has increased significantly in recent years, so much so that the Food and Agriculture Organization (FAO) has predicted an increase in the worldwide use of paper and cardboard from the 210 million tons of 1988 to about 350 million tons by 2010 [27]. Paper consumption in the world in 2004 amounted to an average 52.45 kg per person per year and was 16.32% higher than in 1991. The current annual production of pulp cannot meet the increasing demand, which continues to grow at a dramatic rate [28, 29]. This steady increase in the demand of paper is gradually leading to a worldwide shortage of wood fiber supplies. The virgin forests from which most of the pulp for paper has been obtained for the past 100 years are shrinking. In addition, environmental and population growth pressures are contributing to long-range changes in forest-land management practices, which reduce the harvest of wood for wood products and for pulp and paper manufacture [30]. One possible solution to this problem lies in the use of annual and nonwood plants [31–35].

A number of nonwood fibers are in use all over the world for making paper and other products. In 1970, pulp from nonwood fibers accounted for only 6.7% of the overall worldwide production. By 1993, the proportion had increased to 10.6% and, sometime in the future, it will be predictably double that of pulp made from woody materials [27, 36]. Straw materials are by far the largest source of nonwood fibers, followed by bagasse and bamboo. Straw was used for the first time as a raw material for paper in 1800, and in 1827, the first commercial pulp mill began operations in the United State using straw [37]. In many countries, straw has been used for paper and board production, and interest in this field continues to grow. This is particularly important in these countries where the pulp wood availability is extremely limited [38]. Wheat and rye straws are used in some European countries, such as Bulgaria, Denmark, Greece, Holland, Hungary, Italy, Rumania, Spain, and Yugoslavia, where pulpwood supplies are limited, and the purchase of wood pulp from outside sources is too expensive to support local paper production. The growing utilization of cereal straws has received the attention of many developing countries, particularly

Algeria, Argentina, China, Egypt, India, Indonesia, Mexico, Pakistan, Sri Lanka, Syria, and Turkey. In these countries, corrugating medium, board, and packaging paper are produced from high-yield unbleached straw pulps; bleached straw pulp is used as a major furnish for fine-quality writing, printing, and other paper grades [39]. The greatest part of this increase is attributed to the developing market economies, especially in Asia [40]. Most of the world's increased use of nonwood plant fibers has been attributed to the tremendous increase in nonwood pulping capacities in China. At present, China produces more than two-thirds of nonwood pulp produced worldwide [41]. Major agriculture residues used in China's pulp and paper industry include wheat straw and bagasse. Straw is a major source of fiber for the paper industry in China, which is mainly due to its ready availability.

The main drawbacks that are considered to limit the use of nonwood fibers are the difficulties in collection, transportation, and storage [42, 43]. Besides, straw contains significant amounts of silica, ranging approximately from 3 to 13.3%, which creates potential problems in conventional chemical recovery systems [39]. Despite these drawbacks, the use of straw shows potential as a means of addressing the shortage of raw materials for paper manufacture. Furthermore, the production of pulp from nonwood resources has many advantages such as easy pulping capability, excellent fibers for the special types of paper, and high-quality bleached pulp. Finally, we should note that the analysis of fiber morphology and chemical composition of plant material has been useful in searching for candidate fiber crops. This has provided an indication of the papermaking potential of various species [44]. Morphological characteristics, such as fiber length and width, are important in evaluating the pulp quality of fibers [45], and the chemical composition of the candidate plant gives an idea of the feasibility of using the plant as a raw material for papermaking.

The rapidly growing demand for energy, a dwindling and unstable supply of petroleum, and the emergence of global warming by the use of fossil fuels have rekindled a strong interest in pursuing alternative and renewable energy sources [46]. Biomass as a renewable resource has received more interest. The recovery of energy from biomass has centered thermochemical and biochemical conversion processes. Mechanical extraction (with esterification) is the third technology for producing energy from biomass, for example, rapeseed methyl ester (RME) biodiesel [47]. Within thermochemical conversion, four process options are available: combustion, pyrolysis, gasification, and liquefaction. Biochemical conversion encompasses two process options: digestion (production of biogas, a mixture of mainly methane and carbon dioxide) and fermentation (production of ethanol) [47].

Among the thermochemical processes, pyrolysis has received an increasing attention because the process

conditions may be optimized to produce high energy density pyrolytic oils in addition to the derived char and gas. Gasification—the process of converting carbonaceous materials into gaseous products using media such as air, oxygen, or steam—has been suggested as a cleaner alternative to the combustion of low-density materials such as hulls and straw [48, 49]. The chemical composition of straw feedstocks places specific demands on thermal conversion technologies because alkali, silica, chlorine, and sulfur constituents in straw contribute to slag accumulation and corrosion in many previously tested reactors [50]. The process of biomass liquefaction is very complex; the micellar-like broken down fragments produced by hydrolysis are degraded to smaller compounds by dehydration, dehydrogenation, deoxygenation, and decarboxylation. These compounds once produced, rearrange through condensation, cyclization, and polymerization, leading to new compounds [51].

Ethanol derived from biomass, one of the modern forms of biomass energy, has the potential to be a sustainable transportation fuel, as well as a fuel oxygenate that can replace gasoline [52]. The world ethanol production in 2001 was 31 gigaliters (GL) [53]. The major producers of ethanol are Brazil and the United State, which account for about 62% of the world production. The major feedstock for ethanol in Brazil is sugarcane, whereas corn grain is the main feedstock for ethanol in the United State [54]. However, in recent years, lignocellulosic ethanol production has become attractive because the nonfood portion of the plant can be used to produce ethanol, and there is no competition for feedstock with the food industry. Extensive research and development programs have been initiated worldwide to convert lignocellulosic biomass, such as agricultural residues, forestry wastes, waste paper, and energy crops, which has long been recognized as a potential sustainable source of sugars for biotransformation into biofuels and value-added bio-based products [55, 56]. The

global annual potential bioethanol production from the six major crop residues (corn stover, barley straw, oat straw, rice straw, wheat straw, and sorghum straw) and sugarcane bagasse is estimated by Kim and Dale in 2004 (Table 1.1) [54]. Furthermore, lignin-rich fermentation residue, which is the coproduct of bioethanol made from crop residues and sugarcane bagasse, can potentially generate both 458 terawatt-hours (TWh) of electricity (about 3.6% of world electricity production) and 2.6 Exajoule (EJ) of steam.

Unfortunately, lignocellulosic biomass is highly recalcitrant to biotransformation, both microbial and enzymatic, which limits its use and prevents economically viable conversion into value-added products. Himmel et al. [55] emphasized that natural factors such as the following contribute to the recalcitrance of lignocellulosic feedstock to chemicals or enzymes: the degree of lignification [57], the structural heterogeneity and complexity of cell-wall constituents such as microfibrils and matrix polymers [58], the challenges for enzymes acting on an insoluble substrate [59], the inhibitors to subsequent fermentations that exist naturally in cell walls or are generated during conversion processes [60], and the crystalline cellulose core of cell-wall microfibrils [61]. In the context of the biorefinery, these chemical and structural features of biomass affect liquid penetration and/or enzyme accessibility and activity and, thus, conversion costs. The conversion of lignocellulosic materials into biofuels typically includes three steps: (1) pretreatment of lignocellulose to enhance the enzymatic or microbial digestibility of polysaccharide components; (2) hydrolysis of cellulose and hemicellulose to fermentable reducing sugars; and (3) fermentation of the sugars to liquid fuels or other fermentative products [56, 62, 63]. Up to now, hydrolysis of lignocellulose to monosaccharides is usually catalyzed either by enzymes or by acid catalysts under heterogeneous conditions [64], and none of the known methods is yet cost-effective for large-scale applications [65]. As a result, effective pretreatment strategies are

**TABLE 1.1** Potential Bioethanol Production from the Crop Residues and Sugarcane Bagasse [54]

Potential bioethanol production (GL)	Africa	Asia	Europe	North America	Central America	Oceania	South America	Subtotal
Corn stover		9.75	8.23	38.40		0.07	2.07	58.60
Barley straw		0.61	13.70	3.06	0.05	0.60	0.09	18.10
Oat straw		0.07	1.79	0.73	0.009	0.12	0.06	2.78
Rice straw	5.86	186.8	1.10	3.06	0.77	0.47	6.58	204.60
Wheat straw	1.57	42.6	38.90	14.70	0.82	2.51	2.87	103.80
Sorghum straw			0.10	1.89	0.31	0.09	0.41	2.79
Bagasse	3.33	21.3	0.004	1.31	5.46	1.84	18.10	51.30
Subtotal	10.8	261.0	63.8	63.2	7.42	5.70	30.20	442.00

necessary with the purpose of removing lignin and hemicelluloses, reducing cellulose crystallinity, and increasing the porosity of the materials. Different pretreatment methods have been investigated for different materials [66], but they must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes; and (4) be cost-effective [56]. Himmel et al. [55] mentioned that although biofuel production has been greatly improved by new technologies, there are still challenges that need further investigations.

In addition to feed, paper, and energy, other mainly attractive applications for straw are bio-based products, such as wood-based materials, biodegradable plastics, and adsorbents. Since the 1980s, depletion of the world's forests has steadily forced up the price of wood and wood-based materials [67]. In recent years, it has been difficult to obtain solid woods, and this causes problems for wood-based industry. Agricultural residues offer a great promise and new challenges as a replacement for wood and engineered wood products; rice straw and wheat straw can be easily crushed to chips or particles, which are similar to wood particle or fiber, and may be used as substitutes for wood-based raw materials [68]. Wheat straw, for example, offers desirable geometric and mechanical attributes for replacement of wood in cement-bonded particleboard [69]. Rice straw-wood particle composite boards and rice straw-waste tire particle composite boards are successfully manufactured as insulation boards, using the method used in the wood-based panel industry [68, 70].

Recently, because of the growing environmental awareness and ecological concerns and new legislations, composite industries are seeking more eco-friendly materials for their products [71]. Biodegradable plastics and bio-based polymer products based on annually renewable agricultural and biomass feedstock can form the basis for a portfolio of sustainable, eco-efficient products that can compete and capture markets currently dominated by products based exclusively on petroleum feedstock [72]. Worldwide production of biodegradable plastics grew five-fold between the years 1996 and 2001. There is ample room for market growth, as global production of biodegradable plastics in the year 2001 was approximately 8% of that of petroleum-derived plastics in the same year [3, 73]. It was found that tensile and flexural properties of the agro-residue-filled composites showed that they could be used as an alternative to wood-fiber-filled composites [71].

Water pollution is one of the most serious environmental problems faced by the modern society [74]. Among various pollutant sources, the pollutions from heavy metals, dyes, and oil are serious. Toxic heavy metal ions get introduced to the aquatic streams by means of various industrial activities such as mining, refining ores, fertilizer industries, tanneries,

batteries, paper industries, and pesticides and poses a serious threat to environment [75–77]. Effluents discharged from dyeing industries are highly colored, of low biochemical oxygen demand (BOD), and of high chemical oxygen demand (COD). Disposal of this colored water into receiving waters can be toxic to aquatic life [78, 79]. The dyes upset the biological activity in water bodies. They also pose a problem because they may be mutagenic and carcinogenic [80, 81] and can cause severe damage to human beings, such as dysfunction of kidney, reproductive system, liver, brain, and central nervous system [82]. It is believed that traditional treatment processes of waste streams contaminated with metals and dye have their own inherent limitations such as less efficiency, sensitive operating conditions, and production of secondary sludge, and further the disposal is a costly affair [83]. Among many new technologies, utilizing plant residues as adsorbents for the removal of metal ions and dyes from wastewater is a prominent technology [84, 85]. The promising agricultural waste materials are used in the removal of metal ions and dyes either in their natural form or after some physical or chemical modification. Another powerful technology is the adsorption of heavy metals and dyes by activated carbon for treating wastewater [86–87]. However, the high cost of activated carbon and its loss during the regeneration restricts its application. Therefore, there is a need to search for an effective adsorbent for economical wastewater treatment. A wide variety of activated carbons have been prepared from agricultural residues such as corn straw [88], wheat straw [88], rice straw [89, 90], bagasse [91, 92], cotton stalk [93], coconut husk [94], and rice husks [95]. Due to their low cost, after these materials have been expended, they can be disposed of without expensive regeneration.

Oil is one of the most important energy and raw material source for synthetic polymers and chemicals. In the recent years, tremendous increases of accidental and intentional oil discharges have occurred during production, transportation, and refining [96]. Spilled oil causes immense environmental damage unless it is removed as quickly as possible. One of the most economical and efficient means for the removal of spilled oil from either land or sea is the use of adsorbents [97]. Usually, oil-adsorbent materials can be categorized into three major classes: inorganic mineral products, organic synthetic products, and organic vegetable products [96]. Mineral products include perlite, vermiculites, sorbent clay, and diatomite. These materials do not show adequate buoyancy retention and their oil sorption capacity is generally low [98]. Synthetic sorbents such as polypropylene and polyurethane are the most commonly used commercial sorbents in oil-spill cleanup, due to their oleophilic and hydrophobic characteristics [97]. A disadvantage of these materials is that they degrade very slowly as compared to the mineral or vegetable products. The limitations of the inorganic mineral products and organic synthetic products have led to the recent interest in developing alternative



materials, especially biodegradable ones such as natural agro-based products. Sugarcane bagasse, rice straw, wheat straw, barley straw, kenaf, kapok, cotton, and wool fibers have been used as sorbents in oil-spill cleanup [96, 97, 99–106]. It should be noted that acetylation has been the most widely used and successful chemical modification. Because acetyl groups are more hydrophobic than hydroxyl groups, replacing some of the hydroxyl groups with acetyl groups reduces the hydrophilic property of the cell-wall polymers [107].

It is noticeable that all above-mentioned methods to utilize straw were used in the entire form rather than in as separate components. Straw consists mainly of three groups of organic compounds—cellulose, hemicelluloses, and lignin. In addition to these three main constituents, straw contains various other organic compounds including small amounts of protein, small quantities of waxes, sugars and salts, and insoluble ash. If the valuable compounds of straw could be extracted or separated from straw, many chemicals may be used [1, 6, 108]. The application of straw for chemicals or raw materials after the valuable compounds of the straw have been isolated will be discussed in Chapters 3–6. Chapter 2 will detail the recent studies of structure, ultrastructure, and chemical composition of straw, which will continue to provide new insights into biomass conversion. Many potential applications of straw as novel materials for industries will be discussed in Chapter 7.

In a word, before utilizing it well, we should have a good understanding of straw. This book will lead us to reach the goal of knowing the real identity of straw and producing cost-competitive chemicals or raw materials from straw. With the development of plant science and straw chemistry, we could reasonably believe that straw will play an important role in our lives in the future.

## REFERENCES

- [1] A.R. Staniforth, Cereal straw, Oxford University Press, Oxford, 1979.
- [2] J.W. Lawther, R.C. Sun, A. Goodwin, J. Hague, D. Hlynianski, C. Loxton, W. Rankenhohn, Multi use approach to cereal straw fractionation using thermomechanical pulping, Draft final report, The BioComposites Centre, University of Wales, Bangor, UK, 1996.
- [3] G. Lesoing, I. Rush, T. Klopfenstein, J. Ward, Wheat straw in growing cattle diets, *J. Anim. Sci.* 51 (1980) 257–262.
- [4] Y. Matsumura, T. Minowa, H. Yamamoto, Amount, availability, and potential use of rice straw (agricultural residue) biomass as an energy resource in Japan, *Biomass Bioenerg.* 29 (2005) 347–354.
- [5] O. Theander, Review of straw carbohydrate research, in: R.D. Hill, L. Munck (Eds.), *New Approaches to Research on Cereal Carbohydrates*, Elsevier Science Publishers B. V., Amsterdam, 1985, pp. 217–230.
- [6] M. Stacey, Straw as a potential raw material for chemicals, *Agr. Progress* 51 (1976) 69–75.
- [7] V.L. Kossila, Location and potential feed use, in: F. Sundstol, E. Owens (Eds.), *Straw and Other Fibrous By Products as Feed*, Elsevier, Amsterdam, 1984, pp. 4–24.
- [8] E.R. Ørskov, The role of cereal grain and straw in ruminant feeding, in: R.D. Hill, L. Munck (Eds.), *New Approach to Research on Cereal Carbohydrates*, Elsevier, Amsterdam, 1985, pp. 383–394.
- [9] M.G. Jackson, The alkali treatment of straws, *Anim. Feed Sci. Tech.* 2 (1977) 105–130.
- [10] A.J. Baker, Effect of lignin on the in vitro digestibility of wood pulp, *J. Anim. Sci.* 36 (1973) 768–771.
- [11] D.C. Tomlin, R.R. Johnson, B.A. Dehority, Relationship of lignification to in vitro cellulose digestibility of grasses and legumes, *J. Anim. Sci.* 24 (1965) 161–165.
- [12] Y.W. Han, A.W. Anderson, The problem of rice straw waste: a possible feed through fermentation, *Econ. Bot.* 28 (1974) 338–344.
- [13] I.M. Morrison, The degradation and utilization of straw in the rumen, in: E. Grossbard (Ed.), *Straw Decay and its Effect on Disposal and Utilization*, Wiley, Chichester, 1979, pp. 237–245.
- [14] B. Piatkowski, S. Nagel, Results of chewing and ruminant activity of cows fed rations of cereal straw of various physical forms and after chemical treatment with sodium lye, *Arch. Tierernähr.* 25 (1975) 575–582.
- [15] O. Theander, P. Åman, Chemical composition of some forages and various residues from feeding value determination, *J. Sci. Food Agr.* 31 (1980) 31–37.
- [16] B. Sahoo, M.L. Sarawat, N. Haque, M.Y. Khan, Influence of chemical treatment of wheat straw on carbon, nitrogen and energy balance in sheep, *Small Ruminant Res.* 44 (2002) 201–206.
- [17] N.A. Todorov, Recent developments in animal nutrition research in Eastern Europe, *J. Anim. Sci.* 40 (1974) 1284–1299.
- [18] F. Sundstol, E. Coxworth, D.N. Mowat, Improving the nutritive value of straw and other low quality roughages by treatment with ammonia, *World Anim. Rev.* 26 (1978) 13–21.
- [19] E.A. Orden, K. Yamaki, T. Ichinohe, T. Fujihara, Feeding value of ammoniated rice straw supplemented with rice bran in seep: I. Effect on digestibility, N retention, and microbial protein yield, *Asian Austral. J. Anim.* 13 (2000) 490–496.
- [20] A.M.A. Fadel Elseed, J. Sekine, M. Hishinuma, K. Hamana, Effects of ammonia urea plus calcium hydroxide and animal urine treatments on chemical composition and in sacco degradability of rice straw, *Asian Austral. J. Anim.* 16 (2003) 368–373.
- [21] T.J. Klopfenstein, Chemical treatment of crop residues, *J. Anim. Sci.* 46 (1978) 841–848.
- [22] L.H. Harbers, G.L. Kreitner, G.V. Davis, M.A. Rasmussen, L.R. Corah, Ruminal digestion of ammonium hydroxide treated wheat straw observed by scanning electron microscopy, *J. Anim. Sci.* 54 (1982) 1309–1319.
- [23] L.J. Forney, C.A. Reddy, M. Tien, S.D. Aust, The involvement of hydroxyl radical derived from hydrogen peroxide in lignin degradation by the white rot fungus *Phanerochaete chrysosporium*, *J. Biol. Chem.* 257 (1982) 11455–11462.
- [24] J.K. Glenn, M.A. Morgan, M.B. Mayfield, M. Kuwahara, M.H. Gold, An extracellular  $H_2O_2$  requiring enzyme preparation involved in lignin biodegradation by the white rot basidiomycete *Phanerochaete chrysosporium*, *Arch. Biochem. Biophys.* 242 (1983) 329–341.
- [25] J. Dorado, G. Almendros, S. Camarero, A.T. Martinez, T. Vares, A. Hatakka, Transformation of wheat straw in the course of solid state fermentation by four ligninolytic basidiomycetes, *Enzyme Microb. Tech.* 25 (1999) 605–612.



- [26] S.G. Villas Boas, E. Esposito, D.A. Mitchell, Microbial conversion of lignocellulosic residues for production of animal feeds, *Anim. Feed Sci. Tech.* 98 (2002) 1–12.
- [27] G. Giovannozzi Sermanni, P.L. Cappelletto, A. D'Annibale, C. Perani, Enzymatic pretreatments of nonwoody plants for pulp and paper production, *Tappi J.* 80 (1997) 139–144.
- [28] H.S. Sabharwal, R.A. Young, International agro fiber research initiative, *Tappi J.* 79 (1996) 66–67.
- [29] L. Jimenez, M.J. de la Torre, F. Maestre, J.L. Ferrer, I. Perez, Organosolv pulping of wheat straw by use of phenol, *Bioresource Technol.* 60 (1997) 199–205.
- [30] R. Bruenner, Fiber supply crisis in the Pacific Northwest options & solutions, TAPPI Pacific Section Seminar, Seattle, WA, 1994.
- [31] K.M. Banthia, N.D. Misra, Pulp and paper making from agricultural residues and agro wastes, *Ippta* 18 (1981) 5–13.
- [32] S.R.D. Guha, A.K. Rai, Utilization of agricultural residues for pulp, paper and board, *Ippta* 18 (1981) 83–92.
- [33] L. Jiménez, I. Sánchez, F. López, Characterization of cellulose pulp from agricultural residues, *Tappi J.* 74 (1991) 217–221.
- [34] L. Jiménez, F. López, Characterization of paper sheets from agricultural residues, *Wood Sci. Technol.* 27 (1993) 468–474.
- [35] M. Thykesson, L.A. Sjöberg, P. Ahlgren, Paper properties of grass and straw pulps, *Ind. Crop. Prod.* 7 (1998) 351–362.
- [36] J.E. Atchinson, Nonwood fiber could play major role in future US papermaking furnishes, *Pulp Pap.* 70 (1995) 125–131.
- [37] J.E. Atchison, J.N. McGovern, History of paper and the importance of non wood plant fibers, in: F. Hamilton, B. Leopold, M.J. Kocurek (Eds.), *Pulp and Paper Manufacture. Secondary Fibers and Nonwood Pulping*, TAPPI and CPPA, Atlanta and Montreal, 1987, pp. 1–3.
- [38] O. Theander, P. Åman, Chemical composition of some Swedish cereal straws, *Swed. J. Agr. Res.* 8 (1978) 189–194.
- [39] D.K. Misra, Cereal straw, in: F. Hamilton, B. Leopold, M.J. Kocurek (Eds.), *Pulp and Paper Manufacture, Volume 3, Secondary Fibers and Non wood Pulping*, TAPPI, Atlanta, 1993, pp. 82–93.
- [40] R. Aravamuthan, I. Yayin, Optimizing alkaline pulping of wheat straw to produce stronger corrugating medium, *Tappi J.* 76 (1993) 145–151.
- [41] M. Chandra, Use of non wood plant fibers for pulp and paper industry in Asia: potential in China, Master's degree thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA, 1998.
- [42] G.J. McDougall, I.M. Morrison, D. Stewart, J.D.B. Weyers, J.R. Hillman, Plant fibres: botany chemistry and processing, *J. Sci. Food Agr.* 62 (1993) 1–20.
- [43] M.S. Ilvessalo Pfäffli, *Fiber atlas: identification of papermaking fibers*, Springer Verlag, Berlin, 1995.
- [44] T.F. Clark, Plant fibers in the paper industry, *Econ. Bot.* 19 (1965) 394–405.
- [45] I.M. Wood, The utilization of field crops and crop residues for paper pulp production, *Field Crop Abstr.* 34 (1981) 557–568.
- [46] S.H. Lee, T.V. Doherty, R.J. Linhardt, J.S. Dordick, Ionic liquid mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis, *Biotechnol. Bioeng.* 102 (2009) 1368–1376.
- [47] P. McKendry, Energy production from biomass (part 2): conversion technologies, *Bioresource Technol.* 83 (2002) 47–54.
- [48] A.A. Boateng, W.P. Walawender, L.T. Fan, C.S. Chee, Fluidized bed steam gasification of rice hull, *Bioresource Technol.* 40 (1992) 235–239.
- [49] A.E. Ghaly, A.M. Al Taweel, A. Ergudenler, Development and evaluation of straw feeding for fluidized bed gasifiers, in: E.N. Hogan (Ed.), *Proceedings of Seventh Bioenergy R&D Seminar*, Ottawa, Ontario 1980, 1989, pp. 287–295.
- [50] T.R. Miles, T.R. Miles Jr., L.L. Baxter, R.W. Bryers, B.M. Jenkins, L.L. Oden, Boiler deposits from firing biomass fuels, *Biomass Bioenerg.* 10 (1996) 125–138.
- [51] A. Demirba, Mechanisms of liquefaction and pyrolysis reactions of biomass, *Energ. Convers. Manage.* 41 (2000) 633–646.
- [52] M. Wang, Greet 1.5 transportation fuel cycle model, Illinois: Argonne National Laboratory, 2000, Available at <http://greet.anl.gov/publications.html>.
- [53] C. Berg, World ethanol production 2001, The distillery and bioethanol network, Available at [http://www.distill.com/world\\_ethanol\\_production.htm](http://www.distill.com/world_ethanol_production.htm)
- [54] S. Kim, B.E. Dale, Global potential bioethanol production from wasted crops and crop residues, *Biomass Bioenerg.* 26 (2004) 361–375.
- [55] M.E. Himmel, S.Y. Ding, D.K. Johnson, W.S. Andey, M.R. Nimlos, J.W. Brady, T.D. Foust, Biomass recalcitrance: Engineering plants and enzymes for biofuels production, *Science* 315 (2007) 804–807.
- [56] Y. Sun, J.Y. Cheng, Hydrolysis of lignocellulosic materials for ethanol production: a review, *Bioresource Technol.* 83 (2002) 1–11.
- [57] D.J. Cosgrove, Growth of the plant cell wall, *Nat. Rev. Mol. Cell Biol.* 6 (2005) 850–861.
- [58] K. Iiyama, T.B.T. Lam, B.A. Stone, Covalent cross links in the cell wall, *Plant Physiol.* 104 (1994) 315–320.
- [59] M.E. Himmel, M.F. Ruth, C.E. Wyman, Cellulase for commodity products from cellulosic biomass, *Curr. Opin. Biotechnol.* 10 (1999) 358–364.
- [60] C.E. Wyman, B.E. Dale, R.T. Elander, M. Holtzapple, M.R. Ladisch, Y.Y. Lee, Coordinated development of leading biomass pretreatment technologies, *Bioresource Technol.* 96 (2005) 1959–1966.
- [61] Y. Nishiyama, P. Langan, H. Chanzy, Crystal structure and hydrogen bonding system in cellulose I beta from synchrotron X ray and neutron fiber diffraction, *J. Am. Chem. Soc.* 124 (2002) 9074–9082.
- [62] M. Galbe, G. Zacchi, Pretreatment of lignocellulosic materials for efficient bioethanol production, *Adv. Biochem. Eng. Biotechnol.* 108 (2007) 41–65.
- [63] Y.H.P. Zhang, S.Y. Ding, J.R. Mielenz, J.B. Cui, R.T. Elander, M. Laser, M.E. Himmel, J.R. McMillan, L.R. Lynd, Fractionating recalcitrant lignocellulose at modest reaction conditions, *Biotechnol. Bioeng.* 97 (2007) 214–223.
- [64] G. Stephanopoulos, Challenges in engineering microbes for biofuels production, *Science* 315 (2007) 801–804.
- [65] C.Z. Li, Q. Wang, Z.B. Zhao, Acid in ionic liquid: an efficient system for hydrolysis of lignocellulose, *Green Chem.* 10 (2008) 177–182.
- [66] N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Lee, M. Holtzapple, M. Ladisch, Features of promising technologies for pretreatment of lignocellulosic biomass, *Bioresource Technol.* 96 (2005) 673–686.
- [67] R.C. Sun, X.F. Sun, Fractional and structural characterization of hemicelluloses isolated by alkali and alkaline peroxide from barley straw, *Carbohydr. Res.* 49 (2002) 415–423.
- [68] H.S. Yang, D.J. Kim, H.J. Kim, Rice straw wood particle composite for sound absorbing wooden construction materials, *Bioresource Technol.* 86 (2003) 117–121.
- [69] P. Soroushian, F. Aouadi, H. Chowdhury, A. Nossoni, G. Sarwar, Cement bonded straw board subjected to accelerated processing, *Cement Concrete Comp.* 26 (2004) 797–802.

- [70] H.S. Yang, D.J. Kim, Y.K. Lee, H.J. Kim, J.Y. Jeon, C.W. Kang, Possibility of using waste tire composites reinforced with rice straw as construction materials, *Bioresource Technol.* 95 (2004) 61–65.
- [71] S. Panthapulakkal, M. Sain, Injection molded wheat straw and corn stem filled polypropylene composites, *J. Polym. Environ.* 14 (2006) 265–272.
- [72] A.K. Mohanty, M. Misra, L.T. Drzal, Sustainable bio composites from renewable resources: opportunities and challenges in the green materials world, *J. Polym. Environ.* 10 (2002) 19–26.
- [73] R.A. Gross, B. Kalra, Biodegradable polymers for the environment, *Science* 297 (2002) 803–807.
- [74] C. Baird, *Environmental chemistry*, W.H. Freeman and Company, New York, 1995.
- [75] M. Friedman, A.C. Waiss, Mercury uptake by selected agricultural products and by products, *Environ. Sci. Technol.* 6 (1972) 457–458.
- [76] A. Celik, A. Demirbas, Removal of heavy metal ions from aqueous solutions via adsorption onto modified lignin from pulping wastes, *Energ. Source.* 27 (2005) 1167–1177.
- [77] D. Sud, G. Mahajan, M.P. Kaur, Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions a review, *Bioresource Technol.* 99 (2008) 6017–6027.
- [78] C.K. Lee, K.S. Low, P.Y. Gan, Removal of some organic dyes by acid treat spent bleaching earth, *Environ. Technol.* 20 (1999) 99–104.
- [79] K. Kadirvelu, C. Brasquet, P. Cloirec, Removal of Cu(II), Pb(II) and Ni(II) by adsorption onto activated carbon cloths, *Langmuir* 16 (2000) 8404–8409.
- [80] S. Papic, N. Koprivanac, A. Metes, Optimizing polymer induced flocculation process to remove the active dyes from wastewater, *Environ. Technol.* 21 (2000) 97–105.
- [81] S. Rajeswari, C. Namasivayam, K. Kadirvelu, Orange peel as an adsorbent in the removal of acid violet 17 (acid dye) from aqueous solutions, *Waste Manage.* 21 (2001) 105–110.
- [82] K. Kadirvelu, M. Palanivel, R. Kalpana, S. Rajeswari, Activated carbon prepared from agricultural by product for the treatment of dyeing wastewater, *Bioresource Technol.* 75 (2000) 25–27.
- [83] S.S. Ahluwalia, D. Goyal, Removal of heavy metals from waste tea leaves from aqueous solution, *Eng. Life Sci.* 5 (2005) 158–162.
- [84] G. McKay, M.E. Geundi, M.M. Nassar, Equilibrium studies during the removal of dyestuffs from aqueous solutions using biogases pith, *Water Res.* 21 (1987) 1513–1520.
- [85] R. Sanghi, B. Bhattacharya, Review on decolorisation of aqueous dye solutions by low cost adsorbents, *Color. Technol.* 118 (2002) 256–269.
- [86] K. Kadirvelu, M. Kavipriya, C. Karthika, M. Radhika, N. Vennilamani, S. Patabhi, Utilization of various agricultural wastes for activated carbon preparation and application for the removal of dyes and metal ions from aqueous solutions, *Bioresource Technol.* 87 (2003) 129–132.
- [87] S.E. Bailey, T.J. Olin, R.M. Bricka, D.D. Adrian, A review of potentially low cost sorbents for heavy metals, *Water Res.* 33 (1999) 2469–2479.
- [88] M. Lanzetta, C. Di Blasi, Pyrolysis kinetics of wheat and corn straw, *J. Anal. Appl. Pyrol.* 44 (1998) 181–192.
- [89] M. Ahmedna, W.E. Marshall, R.M. Rao, Production of granular activated carbons from select agricultural byproducts and evaluation of their physical, chemical and adsorption properties, *Bioresource Technol.* 71 (2000) 113–123.
- [90] G.H. Oh, C.R. Park, Preparation and characteristics of rice straw based porous carbons with high adsorption capacity, *Fuel* 81 (2002) 327–336.
- [91] R.S. Juang, F.C. Wu, R.L. Tseng, Characterization and use of activated carbons prepared from bagasses for liquid phase adsorption, *Colloid. Surface. A* 201 (2002) 191–199.
- [92] M. Valix, W.H. Cheung, G. McKay, Preparation of activated carbon using low temperature carbonisation and physical activation of high ash raw bagasse for acid dye adsorption, *Chemosphere* 56 (2004) 493–501.
- [93] A.E. Putun, N. Ozbay, E.P. Onal, E. Putun, Fixed bed pyrolysis of cotton stalk for liquid and solid products, *Fuel Process. Technol.* 86 (2005) 1207–1219.
- [94] I.A.W. Tan, A.L. Ahmad, B.H. Hameed, Adsorption of basic dye on high surface area activated carbon prepared from coconut husk: equilibrium, kinetic and thermodynamic studies, *J. Hazard. Mater.* 154 (2008) 337–346.
- [95] M.M. Mohamed, Acid dye removal: comparison of surfactant modified mesoporous FSM 16 with activated carbon derived from rice husk, *J. Colloid Interf. Sci.* 272 (2004) 28–34.
- [96] X.F. Sun, R.C. Sun, J.X. Sun, A convenient acetylation of sugarcane bagasse using NBS as a catalyst for the preparation of oil sorption active materials, *J. Mater. Sci.* 38 (2003) 3915–3923.
- [97] H.M. Choi, Needle punched cotton nonwovens and other natural fibres as oil cleanup sorbents, *J. Environ. Sci. Heal. A* 31 (1996) 1441–1457.
- [98] H.M. Chol, R.M. Cloud, Natural sorbents in oil spill cleanup, *Environ. Sci. Technol.* 26 (1992) 772–776.
- [99] X.F. Sun, R.C. Sun, J.X. Sun, Acetylation of sugarcane bagasse using NBS as a catalyst under mild reaction conditions for the production of oil sorption active materials, *Bioresource Technol.* 95 (2004) 343–350.
- [100] X.P. Sun, R.C. Sun, J.X. Sun, Acetylation of rice straw with or without catalysts and its characterization as a natural sorbent in oil spill cleanup, *J. Agr. Food Chem.* 50 (2002) 6428–6433.
- [101] R.C. Sun, X.F. Sun, J.X. Sun, Q.K. Zhu, Effect of tertiary amine catalysts on the acetylation of wheat straw for the production of oil sorption active materials, *C. R. Chimie* 7 (2004) 125–134.
- [102] M. Hussein, A.A. Amer, A. El Maghraby, N.A. Taha, Experimental investigation of thermal modification influence on sorption qualities of barley straw, *J. Appl. Sci. Res.* 4 (2008) 652–657.
- [103] T. Sellers, G.D. Miller, M.J. Fuller, Kenaf core as a board raw material, *Forest Prod. J.* 43 (1993) 69–71.
- [104] X.F. Huang, T.T. Lim, The performance and mechanism of hydrophobic oleophilic kapok filter for oil/water separation, *Desalination* 190 (2006) 295–307.
- [105] T.T. Lim, X.F. Huang, Evaluation of kapok (*Ceiba pentandra* (L.) Gaertn.) as a natural hollow hydrophobic oleophilic fibrous sorbent for oil spill cleanup, *Chemosphere* 66 (2007) 955–963.
- [106] M.M. Radetić, D.M. Jocić, P.M. Jovancić, Z.L. Petrović, H.F. Thomas, Recycled wool based nonwoven material as an oil sorbent, *Environ. Sci. Technol.* 37 (2003) 1008–1012.
- [107] R.M. Rowell, Opportunities for lignocellulosic materials and composites, *ACS Sym. Ser.* 476 (1992) 12–27.
- [108] F.E.I. Deswarte, J.H. Clark, J.J.E. Hardy, P.M. Rose, The fractionation of valuable wax products from wheat straw using CO<sub>2</sub>, *Green Chem.* 8 (2006) 39–42.

# Structure, Ultrastructure, and Chemical Composition

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This chapter mainly covers the anatomical structure, ultrastructure, lignin and polysaccharides distribution, and chemical composition aspects in relationship to the field of grass lignocelluloses. The intent is to summarize the obtained information and make a potential user aware of the opportunities that cell wall organization and composition distribution have to offer. Although wood is the most widely used raw material for industrial utilization, many other plant fiber as well as nonwood plant fibers are utilized. The most important group of these are grass plant. Cereal straws, sugarcane bagasse, and bamboo are important grass fibers that are being used now and will be used for a long time in utilizations of renewable resources. The cell wall of wood and nonwood is a heterogeneous natural nanocomposite of cellulose, lignin, and hemicelluloses, with content, composition, and distribution varying over a wide range [1–4].

## 2.1 ANATOMICAL STRUCTURE

A wooden stem has a continuous growth in the diameter due to the cambium, forming secondary wood (xylem-phloem). The xylem of wood species is characterized by different anatomical structure [5–8]. Unlike wood, as a species of monocots, grasses lack cambium. Instead, grasses have a stem structure with numerous vascular bundles scattered in a ground tissue of parenchyma storing cells, which is surrounded by a strong and dense epidermis.

The stem of grass has two principal functions: support and conduction of nutrients and water. The stems of grasses are hollow or less commonly solid cylinder interrupted at intervals by transverse partitions. The units between the partitions represent the internodes and the transverse septa are at the nodes [9]. The vascular bundles are growing vertically, turning off to set leaves at regular intervals. This regular division into nodes and internodes is typical for all grass stems (Fig. 2.1). Typically, the grass stems have the

internodes developed as a hollow cylinder surrounded by a leaf sheath e.g. straw and bamboo [10]. Sugar cane and maize diverge from most species of the grass family by having a solid stem. The stem is made up of essentially the parenchymatous ground tissue with the vascular bundles embedded in it. Vascular bundles are distributed according to two basic plans. One is the vascular bundles that are in two circles near the periphery of the stem, as in cereal straws (Fig. 2.2 left). The other is vascular bundles that are scattered in the ground parenchyma throughout the cross section of the stem, as in corn, sugarcane, bamboo, and palm (Fig. 2.2 right) [10].

Figure 2.3 shows four basic parts (from outside to inside): epidermis, cortex, vascular bundle, and pith of the grass stem [11]. Epidermis cells arranged in parallel rows are closely packed, and it functions to protect the internal parts of the plant. The walls are thickened and covered with a thin waterproof layer that retains water called the cuticle. Wax is deposited in the cutinized layers, and it makes them impermeable to water. The epidermis contains long cells, short cells, cells of stomata, and bulliform cells. Stomata with guard cells are found in the epidermis for gas exchange. Cortex, the zone between epidermis and the vascular bundles, contains collenchyma and parenchyma cells. The pith occupies the central part of the stem and is composed of thin-walled parenchyma cells often with larger intercellular spaces than you would find in the cortex.

The vascular bundles in the stem of the grass plants vary in number and size, but it shows the same basic structure. Each vascular bundle is composed of xylem and phloem. Xylem is a strengthening and conducting tissue transporting water and solutes. The xylem contains the earlier formed protoxylem, and it later differentiated into metaxylem [12]. The phloem contains thin-walled, unlignified sieve tubes with companion cells. The main function of phloem is transport of photosynthetic products. As shown in Fig. 2.4, the phloem and xylem are surrounded by sheaths of



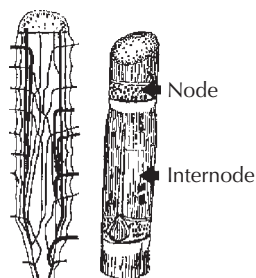


FIGURE 2.1 Longitudinal views of the grass stem [10].

sclerenchyma, including fibers and sclereids [9]. Normally, the sclerenchyma cells in the bundle itself have a small diameter and a thick fiber wall, whereas the extra vascular fibers often have a much larger diameter and a very variable wall thickness. In wheat and rice straw, these fibers occur as coarse bast fibers just inside the epidermal layer (Fig. 2.5) [10]. In bamboo, the main vascular bundle has a typical shape. Each vascular bundle in the central zone of bamboo possesses four sheaths: two sheaths laterally on the either side of the vessels; and two polar, surrounding the phloem and the intercellular spaces. The conducting cells are surrounded by a very dense sclerenchyma tissue, split up by a few rows of parenchyma cells in four distinct parts. The variety of bamboo species is due to the occurrence of extra vascular fibers. These fibers are remarkably different to the ones in the main bundle [10].

## 2.2 ULTRASTRUCTURE

### 2.2.1 Ultrastructure of Wood

#### 2.2.1.1 Fiber Cell Wall Layers

The cell wall structure determines largely the chemical, mechanical, and physical properties of wood. An understanding of cell wall architecture is important for both

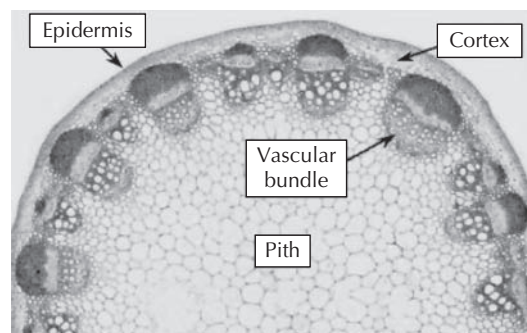


FIGURE 2.3 Light micrograph of cross section of grass stem showing four basic parts [11].

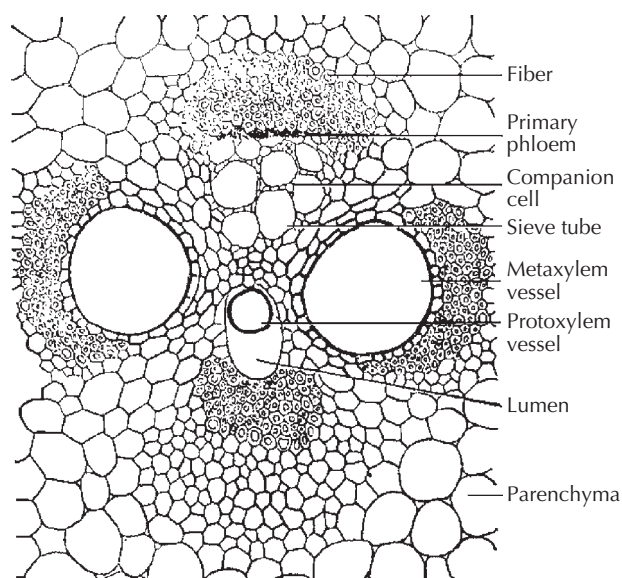


FIGURE 2.4 A cross section of a vascular bundle of bamboo [12].

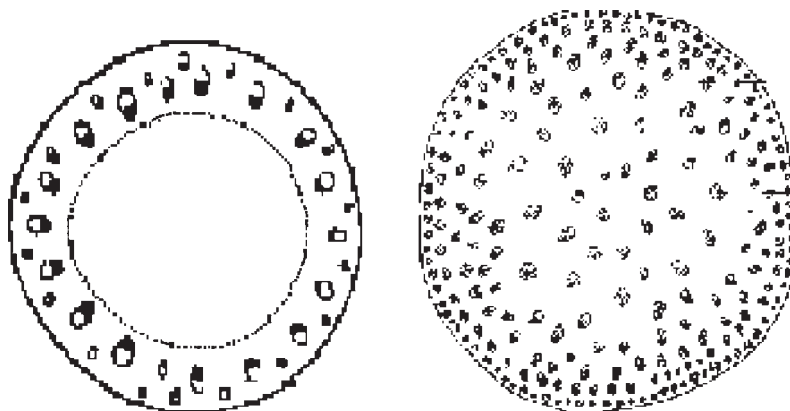
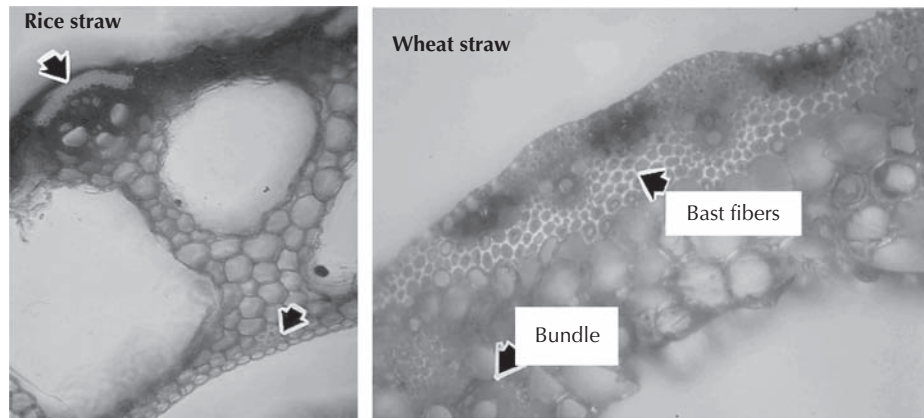


FIGURE 2.2 The cross section of grass stem showing the arrangement of the vascular bundles [10].



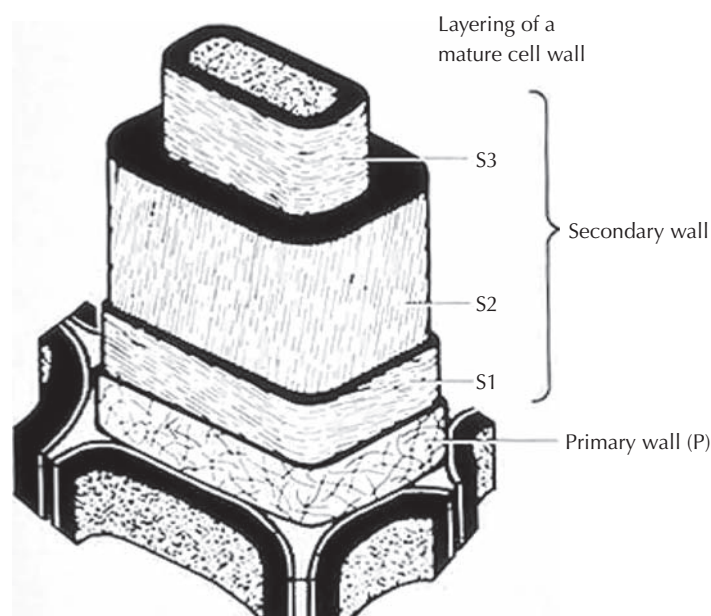
**FIGURE 2.5** Light micrograph of cross section of rice straw and wheat straw [10].

research and technological purposes. Various electron microscopic observations give rise to a model of the construction of wood cell wall, as shown in Fig. 2.6 [13]. The cell wall of normal wood is made up of several layers, namely middle lamella (ML), primary wall (P), and secondary wall (S). Between three or four cell, there is a common area called the cell corner (CC). The ML is a thin layer that glues the individual cells together to form the tissue. Normally, secondary wall is divided into three layers from outside of cell wall to lumen, named outer layer (S1), middle layer (S2), and inner layer (S3). The thickest wall layer is S2. Xu et al. [5] studied the ultrastructure of *C. Korshinskii* and found that normally the fiber cell wall is made up of primary wall, ML, and secondary wall (S1, S2, and S3 layers) (Fig. 2.7).

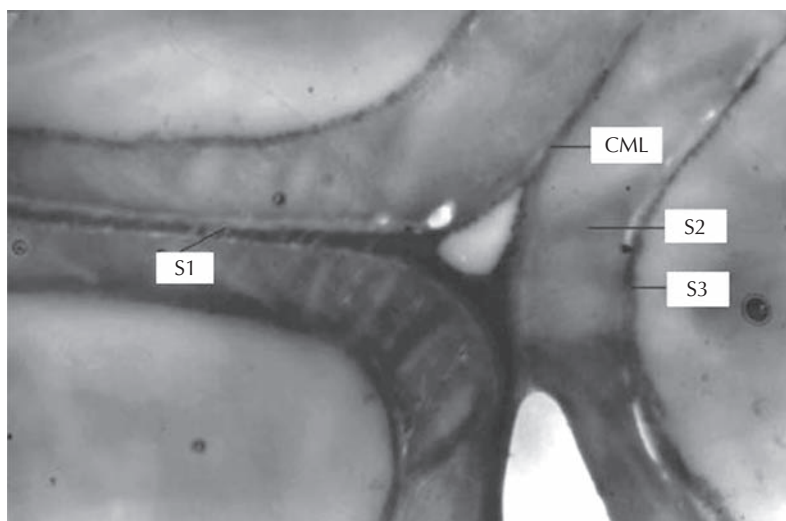
The percentage volume fraction (PVF) of various morphological layers in wood is shown in Tables 2.1 [14]

and 2.2 [15]. Primary wall (P) is a thin layer, only about 0.1–0.2  $\mu\text{m}$  thick, consisting of cellulose, hemicelluloses, pectin, and protein, all completely embedded in lignin. The ML, together with the primary walls on both sides, is often referred to as the compound middle lamella (CML) with higher concentration of hemicelluloses and lignin. The thickness of CML ranges normally from 0.05 to 0.2  $\mu\text{m}$ . The outer layer (S1) is thinner, forms only 10–20% of the total cell wall. The middle layer (S2) forms the main portion of the cell wall taking up 70–90% of the total cell wall. The inner layer (S3) is the thinnest layer, 2–8% of the total cell wall. The change in thickness of S2 is determined by the growing seasons, age, and morphological position. So, the cell wall thickness is mainly depending on S2 layer.

It should be noted that some fiber cell walls are not made up of primary wall and secondary wall. For example, the S3 layer is frequently absent in hardwood. In



**FIGURE 2.6** Simplified structure of a woody cell showing the ML, the primary wall, the outer, middle, and inner layer of the secondary wall [13].



**FIGURE 2.7** Ultrathin sections of the walls of fiber *C. Korshinskii*, which shows the various wall layers: CML, compound middle lamella; S1, secondary wall 1; S2, secondary wall 2; S3, secondary wall,  $\times 10,000$ .

**TABLE 2.1** Average Thickness and Percentage of the Wall Layers in Spruce Tracheids (*Picea Abies*) [14]

Wall layer	Earlywood		Latewood	
	Thickness ( $\mu\text{m}$ )	PVF (%)	Thickness ( $\mu\text{m}$ )	PVF (%)
P	0.09	4.3	0.09	2.1
S1	0.26	12.4	0.38	8.8
S2	1.66	79.0	3.69	85.8
T	0.99	4.3	0.14	3.3
Total wall	2.10		4.30	

compression wood of softwood, the S3 layer is commonly absent, and a general tendency for deep helical fissures occurs in the S2 layer (Fig. 2.8) [16]. The structure of cotton fiber secondary wall is different from that of wood fiber. The winding layer was found to be the first layer of secondary thickening. It differs in structure from either the primary wall or the remainder of the secondary wall. In addition, special structures are also found in the secondary wall of most herbaceous fibers.

The last fibrillar layer at the luminal border should be named tertiary wall (T), and not secondary wall S3, as is sometimes done. It is different from the S3 of the parenchyma cell and the other secondary wall layers. Tertiary wall thickness is quite even within one cell. Differences may occur between different cells, between species, and especially between softwoods and hardwoods. In softwood species, such as *Pinus silvestris* and *Picea abies*, the thickness of the tertiary wall is generally about 700–800 Å. However, it is sometimes considerably thinner [17].

Finally, a thin warty layer may be deposited on the cell wall. In most species, warty layer against the cell lumen exists. The warty layer is one of the major structural features of wood cells found by electron microscopy, which appears to be composed by proteinaceous or lignin-like material (Fig. 2.9) [18]. The warty layer is believed to arise from the extra wall materials and remains of cytoplasm that are deposited on the S3 layer through the plasma membrane [19].

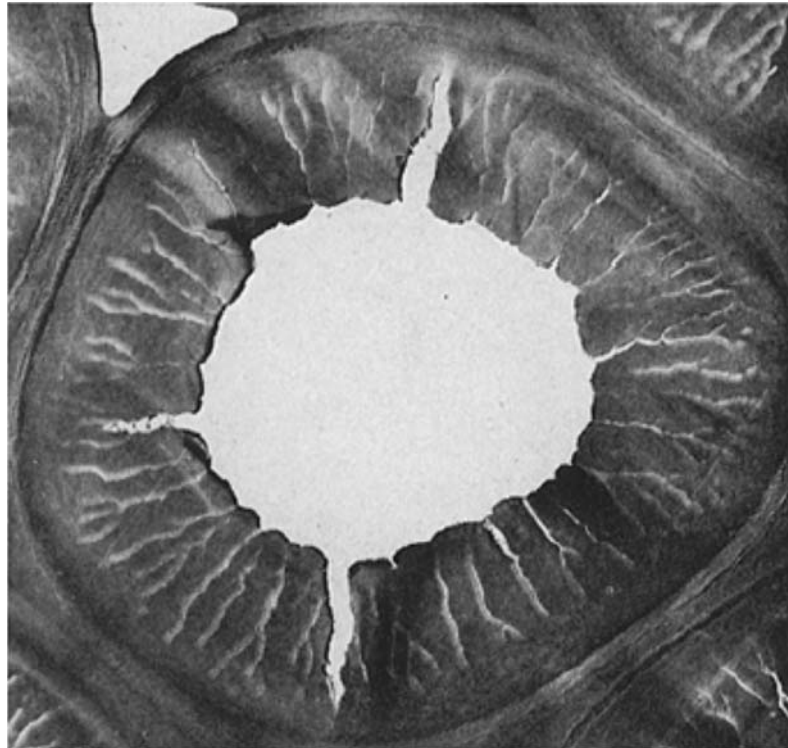
### 2.2.1.2 Microfibrils Organization

Within the cell walls, the microfibrils are cross-linked and stabilized by shorter molecules that make the cellulose microfibrils into a network. The term microfibril angle (MFA) refers to the angle between the direction of helical windings of cellulose microfibrils in the secondary cell wall and the cell's longitudinal axis [20]. MFA plays a crucial role in determining the mechanical behavior of wood. It was found that wood in which the MFA is large has a higher compressive strength, than those have a smaller MFA.



**TABLE 2.2** Average Thickness and Percentage of the Wall Layers in Beech Wood Cells (*Fagus crenata*) [15]

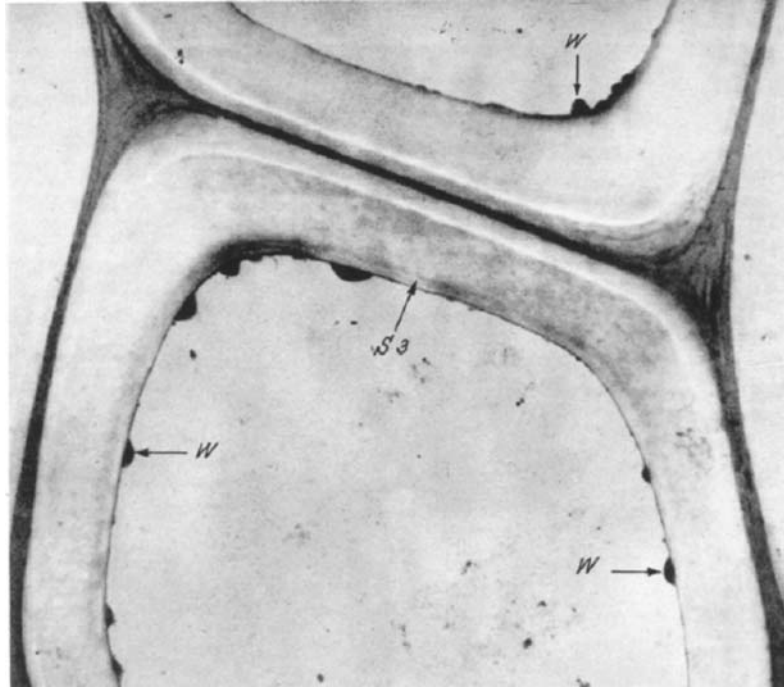
Wall layer	Vessels		Libriform fibers		Fiber tracheids		Longitudinal parenchyma		Ray parenchyma	
	$\mu\text{m}$	%	$\mu\text{m}$	%	$\mu\text{m}$	%	$\mu\text{m}$	%	$\mu\text{m}$	%
P	0.25	25	0.07	1	0.07	5	0.06	4	0.5	27
S1			0.51	10	0.24	16	0.35	21		
S2	0.50	50	4.32	87	0.99	67	0.78	48	0.92	50
S3							0.37	22	0.37	20
T	0.25	25	0.1	2	0.17	12	0.09	5	0.07	3
Total wall	1.00		5.00		1.47		1.65		1.86	

**FIGURE 2.8** Cross section of compression wood of *Picea rubens* Sarg [16].

MFAs vary in different cell wall layers, as shown in Table 2.3. In the primary wall, the microfibrillar network is unstructured, and the microfibril is oriented in a random manner [21]. Wardrop [22] and Harada and Côté [23] proposed a model for the organization of primary wall, in which loose aggregates of microfibrils are oriented approximately longitudinally in the outer part and transversely in the inner part of the primary wall.

The cellulose microfibrils in secondary wall are highly organized and lie broadly parallel to one another within these layers. In S1 layer, the microfibrils lazily wind round

the cell at an angle between  $50^\circ$  and  $75^\circ$  to the cell axis. The S1 layer is rather homogenous with respect to microfibril orientation, the major part of which contains microfibrils orientated perpendicular to the cell axis [24]. Both S and Z lamellae are found in the S1 layer. The microfibrils in the S2 layer are densely packed and steeply inclined, making an angle of only  $10^\circ$ – $30^\circ$  with the cell axis and winding around the cell in the Z direction. In the S3 layer, the orientation of the microfibril changes again to predominantly S helix with the microfibrils steeply inclined relative to the cell axis ( $60^\circ$ – $90^\circ$ ) [25]. The model of microfibril orientation and



**FIGURE 2.9** Transverse section of mature *Pinus radiata* tracheids about 14 cells from the cambium,  $\times 6000$  [18].

**TABLE 2.3** Average Thickness Percentage and Fibrils Orientation of the Wall Layers in Wood

Layer	PVF%	Fibrils orientation	Layer	PVF%	Fibrils orientation
P	>1	Netlike texture	S2	70 90	10 20 (single helix)
S1	10 20	50 70 (S or Z helix)	S3	2 8	60 90 (single helix)

thickness of wood fiber cell wall layers are shown in Fig. 2.10 [25].

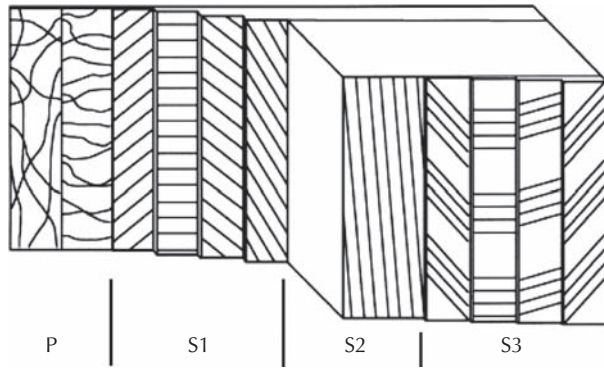
Similarly, Abe et al. [26] suggested that the predominant orientation of microfibrils changed from longitudinal to transverse direction, as the differentiation of tracheids proceeded. First, microfibrils to be deposited in S1 layer were arranged as an S-helix. Then, the orientation of microfibrils changed gradually, with rotation in the clockwise direction as viewed from the lumen side of tracheids, from the outermost to the innermost S1 layer. Microfibrils in S2 layer were oriented in a steep Z-helix with a deviation of less than  $15^\circ$  within the layer. The orientation of microfibrils in S3 layer changed, with rotation in a counterclockwise direction as viewed from the lumen side, from the outermost to the innermost S3 layer. The angle of orientation of microfibrils that were deposited on the innermost S3 layer varied among tracheids from  $40^\circ$  in a Z-helix to  $20^\circ$  in an S-helix.

Although the S2 exhibits a microfibrillar orientation with steep helices, there are transition lamellae on its inner and outer surfaces, as shown in Fig. 2.11 [27]. Several

lamellae in these regions show a gradual shift of MFAs between S1 and S2 and between S2 and S3. However, the gradual shift of MFAs is more abrupt between S2 and S3 than between S1 and S2. As the transition lamellae are relatively thin when compared with the S1 and S2 in the secondary wall, this lamella is generally not detected in transmission electron microscopy (TEM).

In fact, the variations of MFA within the tree depend on species, morphological regions, and environmental condition. MFA has been found to be consistently smaller in latewood cell walls than in those of earlywood in *Eucalyptus nitens* [28] and *Pinus taeda* L. [29]. MFA is also reported to decrease with height in the tree for a particular growth ring from earlywood to latewood. In the same growth ring of different tree height, the MFA of earlywood is large compared to that of latewood. The MFA in the middle of tree height is large than that of near the crown and ground.

A new technique, Raman microscopy, has been successfully developed recently to determine MFA of wood in situ [30]. A bright-field image of a black spruce latewood cell selected for imaging work is shown in



**FIGURE 2.10** A hypothetical model for the orientation of cellulose microfibrils are viewed from the lumen side of tracheids in the cell walls of tracheids of conifers [25].

Fig. 2.12. Raman images of S1 and S2 regions produced using cellulose contribution at 1098 and 2773  $3045\text{ cm}^{-1}$  showed significant differences (Fig. 2.13). The spectra associated with the S2 images indicated the microfibrillar orientation there to be longitudinal, in contrast, the spectra corresponding to the predominantly S1 images indicated that cellulose microfibrils in S1 was oriented approximately  $60^\circ$  to the expanding direction of growing cell.

### 2.2.1.3 Matrix Components

The matrix phase in the cellulosic fiber cell wall consists of lignin and hemicelluloses and any other polymers surrounded the microfibrils. The microfibrils constitute the structural framework and consist essentially of cellulose I [19]. The plant cell wall appears to be made up of cellulose fibrils of about 20–25 nm in width. When the cell wall is disrupted, microfibrils of 3.5 nm in diameter can be observed in electron micrographs. The 3.5 nm structure has come to be known as the basic cellulose crystalline structure (elementary fibril) [31].

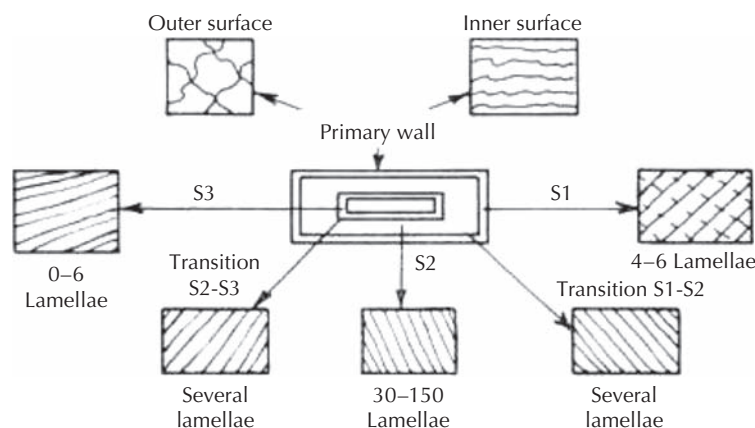
The width of cellulose microfibrils is reported to vary in different cellulose materials. For instance, *Valonia* cellulose

microfibrils, being about 20 nm in width, are five times larger than those of wood. Browning [32] suggested that the width of fibrils is between 20 and 40 nm, and microfibrils of 10 nm diameter can be observed by means of electron microscopy. Hanna and Côté [33] indicated that elementary fibrils vary in size, with the smallest (1.0–1.5 nm width) occurring in the cambial regions. According to Fengel and Wegener [1], several elementary fibrils with an average thickness of 3.5 nm can associate with one another to form cellulose crystallites whose dimensions depend on the origin and treatment of the sample. Four of these basic crystalline aggregates are then held together by a monolayer of hemicelluloses, generating 25 nm wide thread-like structures that are enclosed in a matrix of hemicelluloses and protolignin. The natural composite that results from this close association is referred to as cellulose microfibril (Fig. 2.14) [1]. Kerr and Goring [34] developed a model consisting layers of cellulose-polyoses blocks interrupted in radial and tangential direction by lignin-polyoses blocks. Width of cellulose fibril is 3.5 nm in radial direction and 2 nm in tangential direction (Fig. 2.15).

The model of cell wall matrix organization is shown in Fig. 2.16 [35]. As the structural framework, cellulose is organized into microfibrils, each measuring about 3–6 nm in diameter and containing up to 36 glucan chains having thousands of glucose residues. A microfibril's crystalline and amorphous cellulose core is surrounded by hemicelluloses. In addition to cross-linking individual microfibrils, hemicellulose in secondary cell walls forms covalent associations with lignin. Lignin is the encrusting substance binding the wood cells together and giving rigidity to the cell wall.

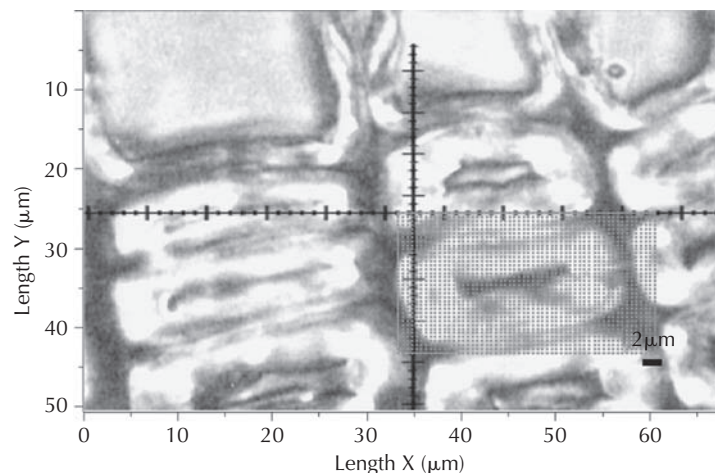
### 2.2.2 Ultrastructure of Grass

Just like wood tracheids, the wheat straw fibers consist of ML, primary wall, S1, S2, and S3, as shown in Fig. 2.17 [36]. The results of various morphological layers thickness in wheat straw together with those obtained from spruce are

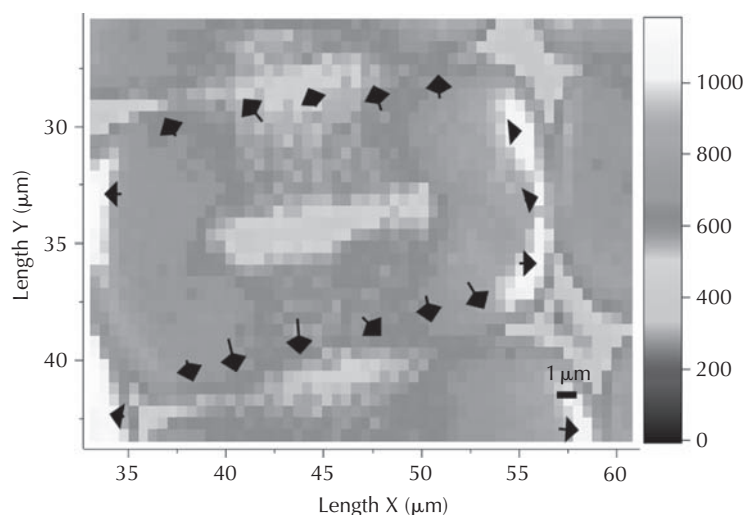


**FIGURE 2.11** Arrangement of microfibrils in various wall lamellae of *Pinus radiata* showing primary wall and transition lamellae [27].





**FIGURE 2.12** Bright field image of partially delignified black spruce wood tissue [30]. (see color plate)



**FIGURE 2.13** Raman images of cellulose spatial orientation and/or distribution in the cell wall [30]. (see color plate)

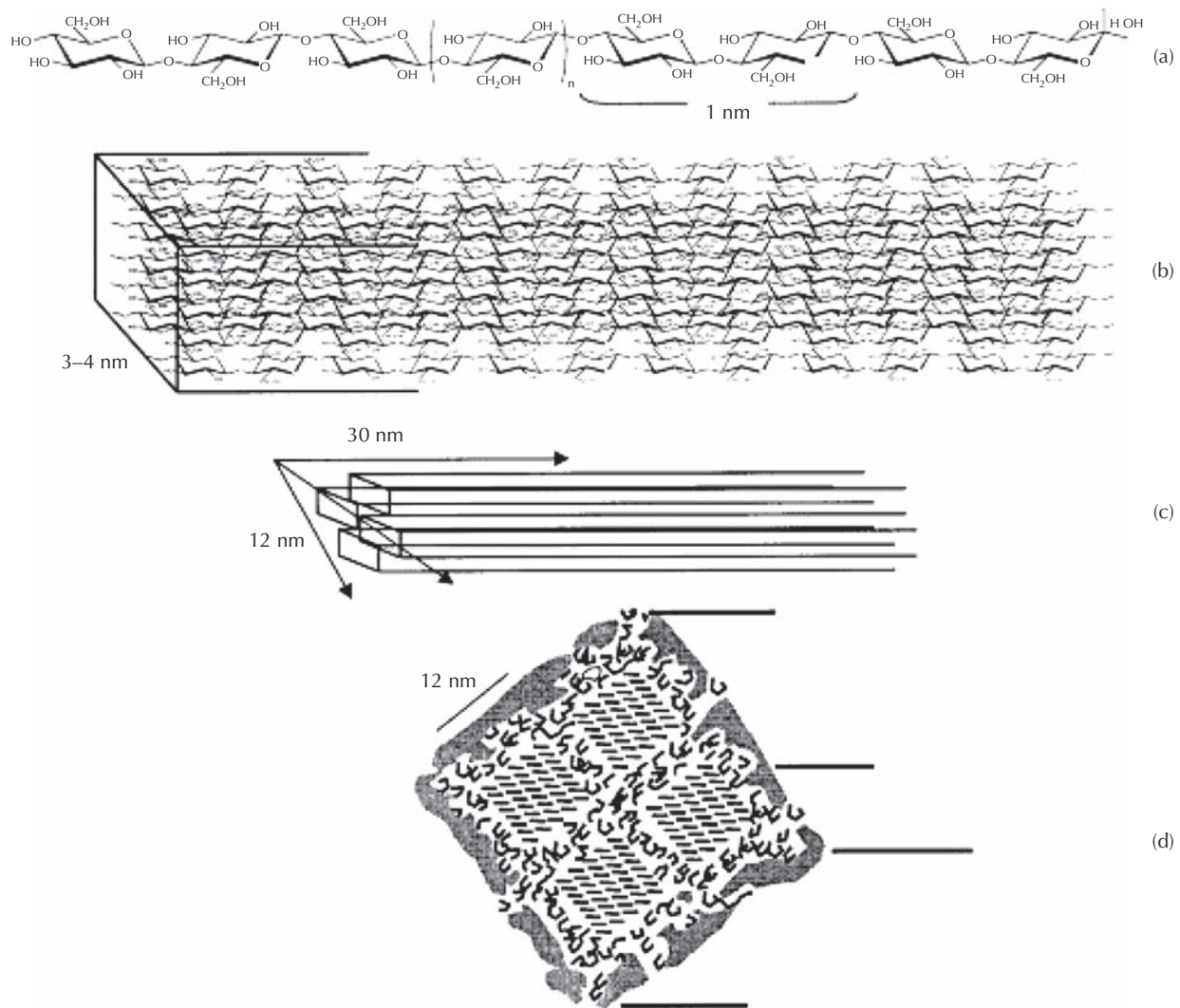
shown in Table 2.4. It is clear that S1 layer of wheat straw fiber is thicker than that of the spruce tracheid. The PVF of ML and CC in wheat straw fiber is greater than that of spruce tracheid.

The orientation of fibrils in each layer in wheat straw fiber is different. As shown in Fig. 2.18, the fibrils of the primary wall display a netlike texture. On the other hand, the fibrils of S1 layer are oriented helically and almost perpendicular to the fiber axis. The orientation of fibrils in S2 layer is about 20°–30°, as shown in Fig. 2.19. Because the wheat straw fiber has a thick S1 layer in which the fibrils are oriented laterally in cross helix, it makes defibration of wheat straw pulp more difficult. The pits in wheat straw fiber are irregular conical chambers (Fig. 2.20). A warty layer is observed in Fig. 2.20. This is the first discovery of a warty layer in nonwood raw materials [37].

The cell wall of reed is made up of primary wall and secondary wall. However, the secondary wall of reed fiber

is not made up by S1, S2, and S3, and it is quite different from that of wood. According to the studies of Chinese Institute of Pulp and Paper Industry, one-third to one-half of reed fiber was found to be abnormal in the S2 layer [38]. The S2 layer is made up by S2-1, S2-2, and S2-3 from outer layer to inner layer. The orientations of microfibrils varied from 70° to 80° in outer layer to 30°–40° (or parallel to fiber cell axis) in inner layer. In some cases, the S3 layer is absent in secondary wall. The thickness of reed fiber is shown in Table 2.5. As can be seen, the secondary wall of reed fiber A is normally made up of S1, S2, and S3. However, the secondary wall of reed fiber B is made up of S1, S2-1, S2-2, and S3. The thickness of S1 layer both in fiber A and fiber B is greater than that in spruce tracheid. A significant difference between the two types of reed fiber is that S2 layer of fiber B is thicker than that of fiber A.

Similarly, the cell wall of sugarcane bagasse fiber contains primary wall and secondary wall. In sugarcane



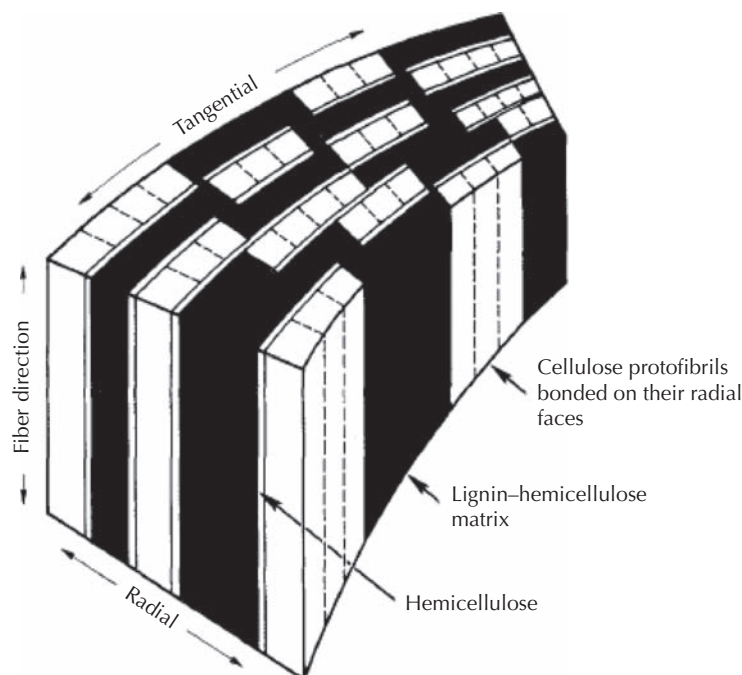
**FIGURE 2.14** Chemical association of the plant cell wall. (a) The cellulose backbone, with an indication the length of its basic structure unit, cellobiose; (b) framework of cellulose chains in the elementary fibril; (c) cellulose crystallite; (d) microfibril cross section, showing strands of cellulose molecules embedded in a matrix of hemicellulose and pectin [1].

bagasse fiber, the secondary wall is alternatively arranged by broad layer and narrow layer, or built up by a number of layers. Lignin concentration is lower in broad layer than that in narrow layer [38] (Fig. 2.21).

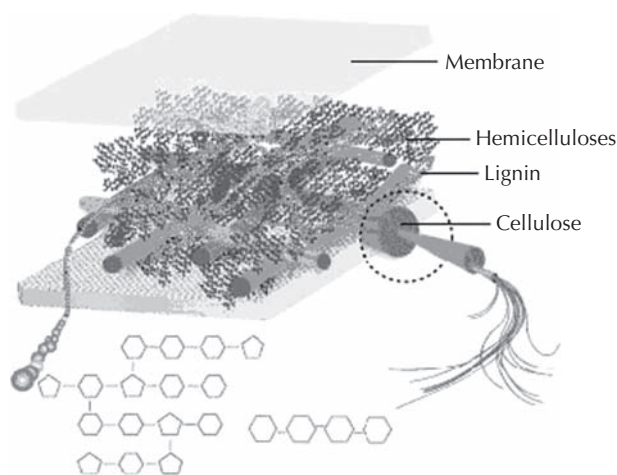
The bamboos constitute an economically important group of grass plants especially in Asia. A majority of the fibers does not possess any obvious layering of their walls. However, thick-walled fibers with distinct layering occur especially in the peripheral region adjacent to the epidermis. In cross sections, these walls are characterized by a regular alternation of broad and narrow lamellae or layers. The number of lamellae varies in the species and a maximum of 18 is found [39].

The fine structure of the fiber wall shows a distinct polylamellate construction (Fig. 2.22). A thin primary wall with the usual meshwork of fibrils is present at the ML. The fibrils in the following outermost lamella exhibit an angle of

approximately  $50^\circ$  to the vertical. Transition lamella of the secondary wall, however, is not always to be observed. The change in the orientation of the fibrils from this into the first broad lamella takes place at an angle of  $35^\circ$ . In the broad lamella, the fibrils are almost longitudinally oriented with a deviation of  $2-5^\circ$  from the cell axis. In the successive broad lamellae, a slight increase in the fibrillar angle occurs: the second inner broad lamella shows  $10-12^\circ$  and the following shows  $10-20^\circ$ . In the narrow lamellae, fibrils show a nearly horizontal orientation of  $85-90^\circ$  that remains almost constant. Characteristically, the fibrils show a slight depression before merging into the fibrils of the next broad lamellar zone. The innermost lamellae near the lumen are mostly thinner than the others (Fig. 2.23). The lamella at the lumen boundary is either of the broad or narrow type with no resemblance to the tertiary wall typical of wood fibers. A warty layer is present sporadically in the fibers [39].



**FIGURE 2.15** Pictorial representation of the proposed interrupted lamella model for the ultrastructural arrangement of lignin, cellulose, and hemicelluloses in the wood cell wall [34].



**FIGURE 2.16** The model of cell wall organization [35]. (see color plate)

A model for the thick-walled bamboo fiber is presented (Fig. 2.24) [39]. The cell wall is built up by the primary wall and the secondary wall transition lamella S0 (S zero, not always present), S1-*l*, S2-*t*, S3-*l*, S4-*t*, S5-*l*, S6-*t*, etc. The affixes *l* and *t* stand for the almost longitudinal and transverse orientation of the cellulose fibrils in the respective lamellae.

The fiber cell walls within the vascular bundles of oil palm frond (OPF), coconut (COIR), banana stem (BS), and pineapple leaf (PALF) were studied [40]. As shown in Fig. 2.25, the layered structure of these four agro fiber wall contained primary wall and secondary wall (S1, S2, and S3) layers. This structure was similar to the wood cell wall

structure that has been proposed by Harada and Côté [23]. The primary wall appeared as a solid boundary of the cell. The ML showed a clear transition to the adjacent primary wall layers. The S1 layer of all fibers was well defined and could be distinguished from the adjoining S2 layer. The thickness of S1 layer in agro fibers was found to be in range of 0.10–0.84  $\mu\text{m}$ . The S2 layer of these agro fibers occupied about 43–78% of the whole wall in thickness, and BS fibers exhibited the thickest S2 layer (1.57  $\mu\text{m}$ ). Thick-walled cells contain a large S2 layer, whereas thin-walled cells have a small S2 layer.

A distinct S3 layer is present in four agro fibers. The great variability in the thickness of the S3 layer occurs. COIR fibers contained a well-developed S3 layer, being the thickest S3 layer (0.089  $\mu\text{m}$ ) among all these fibers. COIR fibers are supposed to have higher mechanical strength, as also proposed by Booker and Sell [41]. Singh et al. [42] suggested that the irregular thickness might be better suited to relieve the pressure of the axial compression force on the tracheid wall than one of uniform thickness.

## 2.3 DISTRIBUTION OF POLYSACCHARIDES AND LIGNIN

### 2.3.1 Polysaccharides Distribution

The distribution of cellulose is probably the easiest to study the various morphological regions of wood. It is well known that ML is, in principle, free of cellulose, but it is



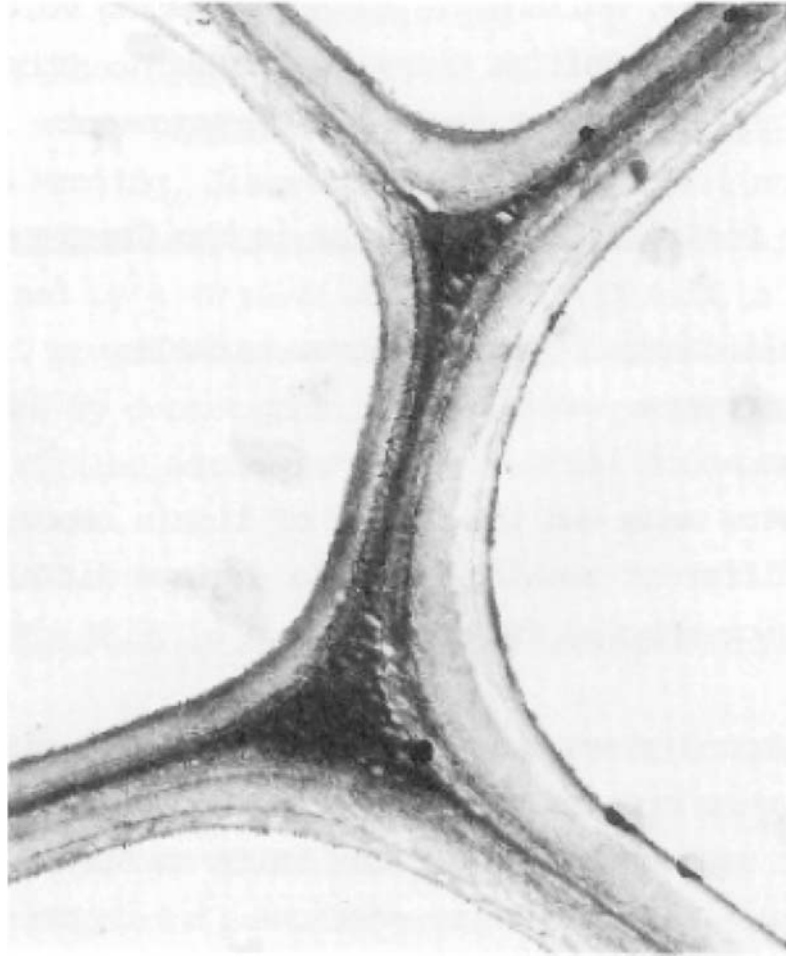


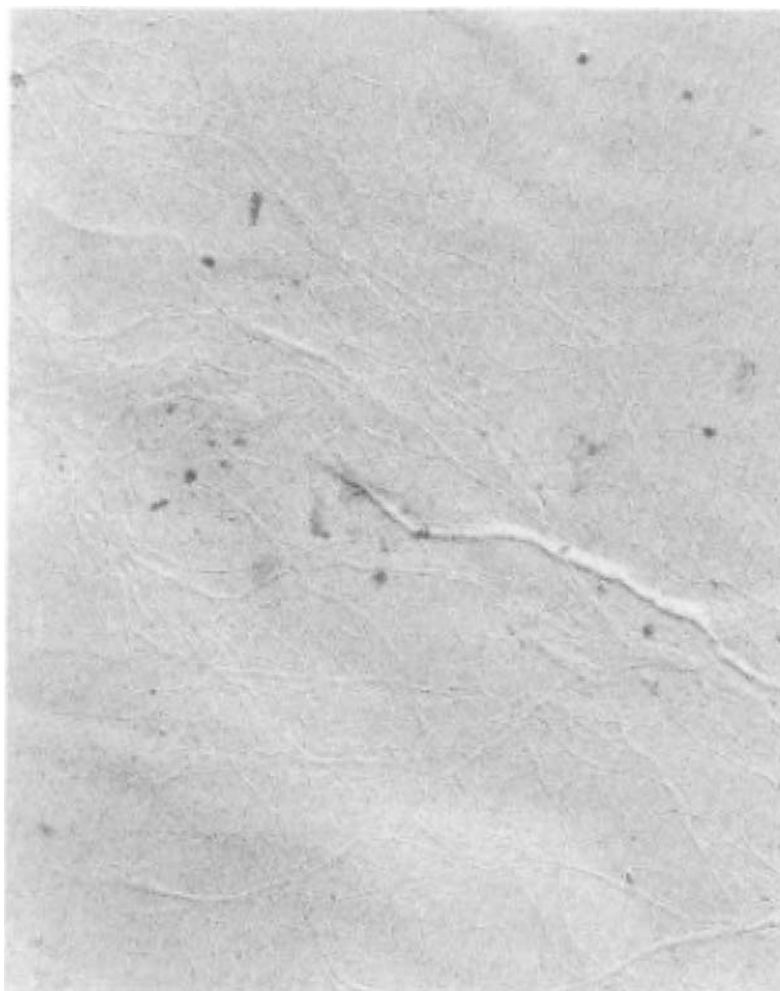
FIGURE 2.17 Transverse section of wheat straw,  $\times 6000$  [36].

**TABLE 2.4** The Thickness and PVF of Various Morphological Layers in Wheat Straw Fiber and Spruce Tracheid [36]

Morphological layers	Wheat straw				Spruce tracheid	
	Thick fiber		Thin fiber			
	Thickness ( $\mu\text{m}$ )	PVF (%)	Thickness ( $\mu\text{m}$ )	PVF (%)	Thickness ( $\mu\text{m}$ )	PVF (%)
ML+P	0.1 0.2	9.3	0.06 0.12	12.3	0.05 0.1	10.2
S1	0.1 0.3	83.5	0.2 0.3	80.7	0.15 0.2	9.9
S2	1.8 2.5		0.5 0.8		0.7 2.0	75.9
S3	0.15 0.3		0.1 0.2		0.1	4.0
CC		5.4		7.0		

mainly composed of lignin, hemicelluloses, and limited pectin substances. In primary wall, microfibrils form a loose structure with high lignin and hemicelluloses consistency. The cellulose concentration in the secondary wall may be higher than that of the primary wall. The microfibrils in the secondary wall, especially in S2 layer, is orientated

regularly with lower lignin and hemicelluloses concentration. In birch, it was indicated by Meier [43] that cellulose content is 41.4% in CML, 49.8% in S1 layer, 48% in outer part of S2, and 60% in inner part of S2 and S3 layers, respectively (Table 2.6). In spruce, however, cellulose content is 33.4% in CML, 55.2% in S1 layer, 64.3% in



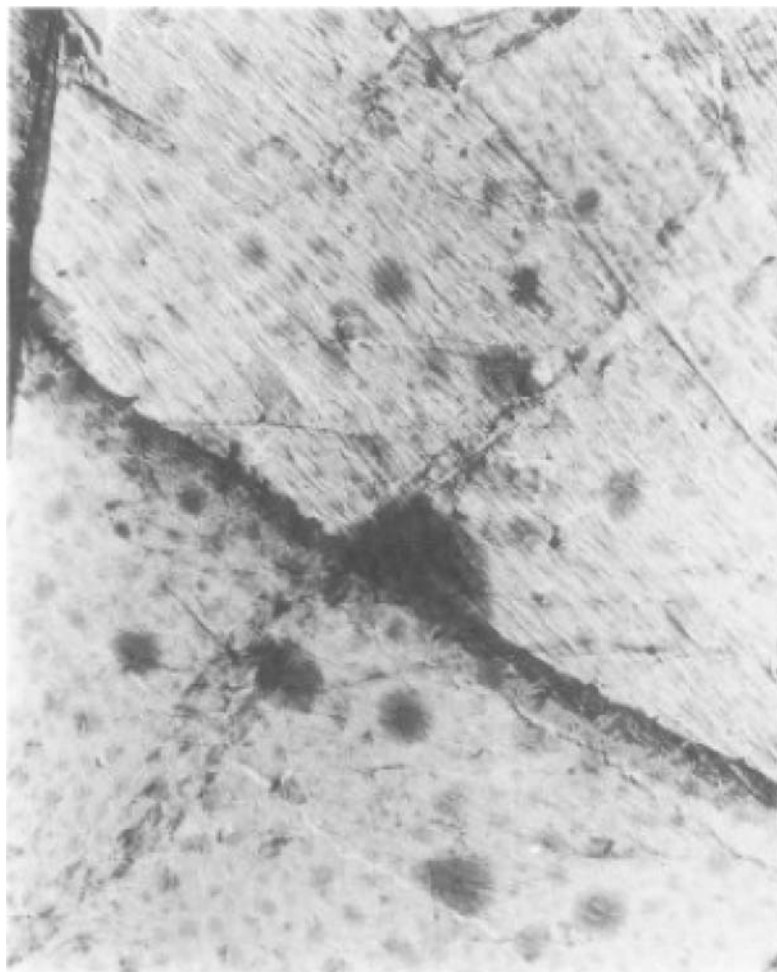
**FIGURE 2.18** Fibril orientation of primary wall (a) and S1 layer of secondary wall (b) in wheat [36].

outer part of S2, and 63.6% in outer part of S2 and S3 layers, respectively. Cellulose distribution in *Pinus Silvestris* has followed a similar pattern to spruce. In summary, cellulose distribution in cell walls has an obviously regular pattern. Cellulose content increases from outer layer to inner layer. The cellulose content is highest in the secondary wall, especially in S2 and S3.

More recently, cellulose distribution in cell wall has been investigated without being disruptive to native-state structures using Raman microscopy [4, 44–47]. The chemical information showed that the cellulose distribution in S2 layer was much more uniform (mostly red/magenta, Fig. 2.26) as compared to that of lignin [4]. High cellulose concentration spots were confined mostly to S2 and S2-S3 regions, while cellulose concentration in CC areas and CML were low. Figure 2.27 shows the typical spectra in cellulose frequency range [4]. The band intensity varied depending on the specific location in the region. Cellulose concentration varied more significant in S2 and S2-S3 regions than that in CC and CML.

The hemicelluloses have not received adequate attention in studies of wood cell walls because the complexity of their structures does not admit easy interpretation within the paradigms of polymer science [48]. Recent studies have shown that the hemicelluloses can participate in regulation of nanoscale architecture of cell wall constituents. They influence the aggregation of celluloses, and they can also influence the pattern of interunit linkages in lignin analogs polymerized in their presence. It is plausible, therefore, to consider the possibility that the hemicelluloses have higher functions than as mechanical coupling agents in a two-phase composite [49].

Unlike cellulose, a study of the distribution of hemicelluloses is difficult. Various methods employed for the direct and indirect demonstration of polysaccharides are periodate-hexamine oxidation method [49, 50], periodic-Schiffs reagent [51], negative staining methods [52, 53], extraction and enzymatic treatment [54, 55], in situ staining with uranyl acetate and lead citrate [56], and autoradiography of pentosans [57].



**FIGURE 2.19** Fibril orientation of S2 layer of wheat straw fiber [36].

Hoffmann and Pammeswaran [58] attempted in situ experiments on holocellulose of sprucewood to detect hemicelluloses. It was observed using scanning electron microscopy (SEM) that the CML and CC were stained into the darkest (highest hemicelluloses concentration), followed by outer part of S1 layer and S3 layer. The lightest color was S2 layer, which indicated that hemicellulose concentration is lowest in this layer. In summary, hemicelluloses distribution in spruce tracheids is higher in ML and S1 layer, but lower in S2 layer (Fig. 2.28) [58]. Table 2.7 shows the content of hemicelluloses in the cell wall layers of *Betula verrucosa*, *Picea abies*, and *Pinus silvestris*. As can be seen, the content of hemicelluloses is the high in CML of these three species.

In S1 of birchwood and sprucewood, Meier [59] found that the glucuronoxylan distribution is higher than that in ML and P. Accordingly, the S1 layer and outer part of S2 layer contain a high amount of glucuronoxylan. The glucuronoarabinoxylan content was found to be high in tertiary walls of sprucewood tracheids. Takabe [60] found that glucuronoarabinoxylan and galactoglucomannan are

rich in the S1 and the outer part of S2 and S3 layer. Parameswaran and Liese [61] indicated that the mannan in sprucewood is high in S1 and tertiary (T) wall layer, while xylan is higher in tertiary wall than S1. In beechwood, the xylan distribution shows a higher concentration in S1 and T and a lower one in S2. According to the various methods employed, it was demonstrated that the middle part of S2 possesses, on the whole, a lower concentration of hemicelluloses than the inner and outer parts of the secondary wall. The S1 and S2 boundary has proved to be high in xylan and mannan content. The hemicelluloses content in the tertiary wall can equally be considered high, as in the S1 layer.

### 2.3.2 Lignin Distribution

The location of the wall component in woody cells has attracted considerable attention for a long time. Among the wall components, lignin ranks first. Lignin has significantly different properties with carbohydrates, such as activity and



**FIGURE 2.20** Pits of wheat straw fiber [36].

solubility. Lignin can react with  $\text{KMnO}_4$ ,  $\text{Cl}_2$ , and  $\text{Br}_2$ . Distribution of lignin in plant cell wall can be analyzed according to these properties. Several techniques have been applied to the study of distribution of lignin and organization of wood components. One of the oldest procedures is selective staining, followed by the study under light microscope [62]. Another method to study lignin distribution was reported using electron microscopy of lignin skeletons. Figure 2.29 shows the electron image of lignin skeletons [63]. After a chemical treatment of wood samples with HF or carbohydrase to dissolve the carbohydrate, the

lignin skeleton of cell wall was remained. The wood samples were then detected by electron microscope after a series of dewatering, embedding, and cutting into ultrathin sections.

Although the aforementioned methods are useful in studying lignin distribution, only qualitative or semiquantitative information of lignin in various morphological regions can be provided. The earlier studies that provided quantitative information of lignin distribution in the secondary wall and CML were performed using ultraviolet (UV) microscopy [64]. UV absorption is a tool used for lignin identification because lignin absorption occurs at 280 nm. The weight concentration of lignin was estimated to be 16% and 73% for the secondary wall and CML of Norway spruce tracheids, respectively. Considerable researches have been undertaken by using UV microscopy in the literature [65, 66].

Of the other methods for quantitative assay of lignin distribution within the cell wall layers, interference microscopy based on the pathways of rays was proposed by Lange and Kjaer [67]. Boutelje [68] refined this technique later. With the development of electron dispersive X-ray analysis (EDXA) combined with SEM and TEM, these studies and values were revisited. Interference microscopy and bromination in conjunction with EDXA [69, 70] were applied to determined lignin distribution in wood and nonwood, in differentiating xylem, and in chips during pulping. The technique is fully quantitative and accurate. The quantitative distribution of lignin has been also studied by means of mercurisation with subsequent TEM- or SEM-EDXA observations [5, 71, 72].

Confocal laser scanning microscopy (CLSM) has been used to study lignin distribution in agricultural fibers [73] and the difference between normal wood and tension wood [6]. Recently, Raman microprobe spectroscopy was used to study the concentration of lignin in the cell walls of black spruce wood in situ [47]. Chemical information from morphological cell wall regions was obtained, and Raman

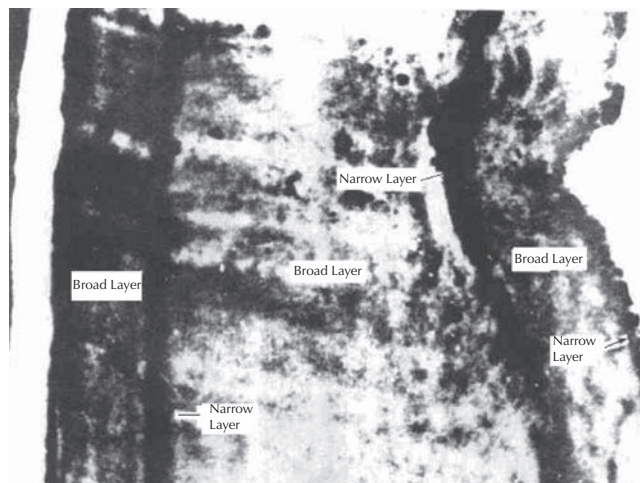
**TABLE 2.5** Thickness of Various Morphological Regions in Reed Fiber and Spruce Tracheid [38]

Morphological regions	Reed fiber A <sup>a</sup>		Reed fiber B <sup>b</sup>		Spruce tracheid	
	Thickness (μm)	%	Thickness (μm)	%	Thickness (μm)	%
CML	0.07	11.9	0.07	7.8	0.05–0.1	10.2
S1	0.3–0.5	31.2	0.2–0.3	16.6	0.15–0.2	9.9
S2-1	0.7–1.0	48.2	0.8–1.4	54.7	0.7–2.0	75.9
S2-2			0.4–0.6	16.3		
S3	0.2–0.3	8.7	0.15–0.2	4.6	0.1	4.0

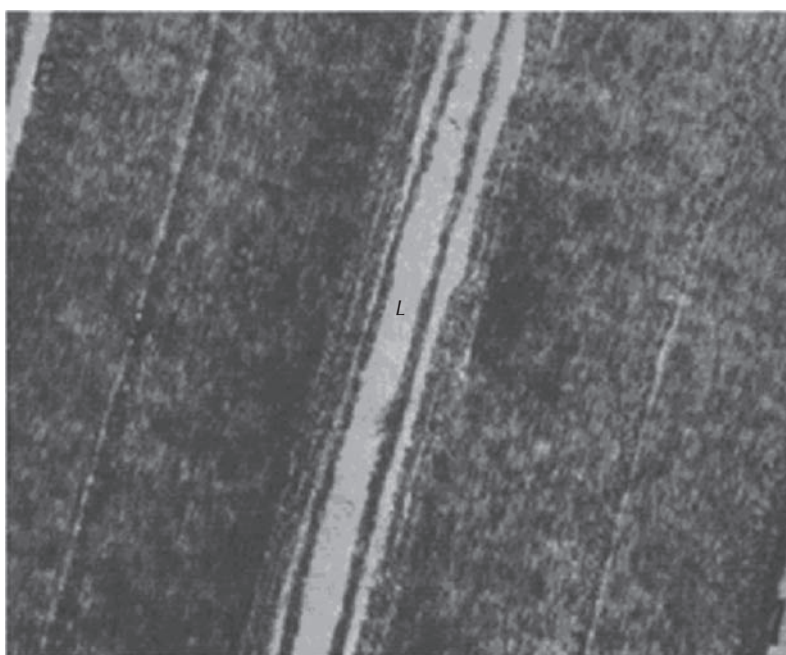
<sup>a</sup>Reed fiber A: the secondary wall of reed fiber is normally built up by S1, S2, and S3.

<sup>b</sup>Reed fiber B: the secondary wall of reed fiber is built up by S1, S2-1, S2-2, and S3.





**FIGURE 2.21** TEM image of cross section of sugarcane bagasse fiber [38].



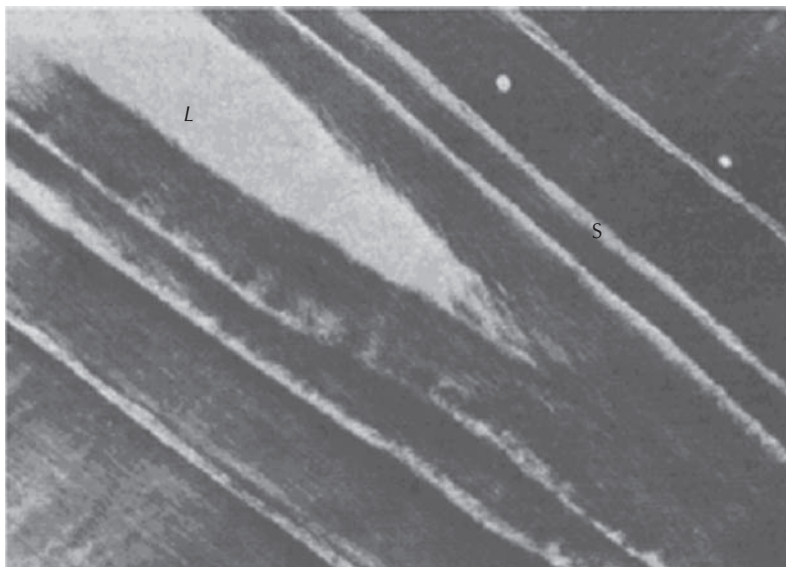
**FIGURE 2.22** Slightly biased longisecton of a delignified fiber wall near to lumen (L) with broad (S l) and narrow (S t) lamellae as well as transitional zones between lamellae. *Dendrocalamus latiflorus*,  $\times 9400$  [39].

images of lignin distribution generated using the  $1600\text{ cm}^{-1}$  band was determined. It was reported that CC lignin concentration was the highest on average. Lignin concentration in CML was not significantly different from that in secondary wall.

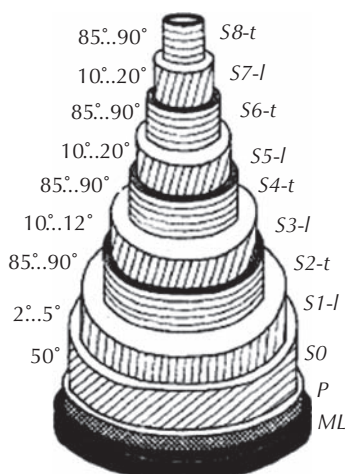
### 2.3.2.1 Lignin Distribution in Wood

A number of early investigations established that the CC is the highest lignified in cell walls. Lignin distribution in different morphological region of spruce was studied by Wood and Goring [66] using UV microscopy (Fig. 2.30). As shown in Table 2.8, the results indicated that lignin

concentration is the highest in ML and CC. However, the lignin content is not high according to their low tissue volume. Although lignin concentration in secondary wall is lower, the lignin content is the highest due to its high tissue volume. The CC is often more highly lignified than the CML. The results from autoradiographic investigation also showed that the lignin distribution in CML is higher than the secondary wall [74]. The CML region of the cell typically contains more than 50% lignin concentration (w/w), while the S2 region contains about 20% [75]. Table 2.9 shows the distribution of lignin in loblolly pine (*Pinus taeda* L.) tracheids as determined by bromination coupled with SEM-EDXA [76]. As can be seen, the concentration in



**FIGURE 2.23** Longisection through a thick walled delignified fiber from primary wall to primary wall (P). Note the difference in thickness of lamellae towards the lumen tendency for a decrease in width of lamellae. *Phyllostachys edulis*,  $\times 4000$  [39].



**FIGURE 2.24** Model of polylamellate structure of a thick walled bamboo fiber [39]. Numbers on the left indicate fibril angle, and letters on the right indicate terminology of wall lamellae.

S2 is 0.20 g/g for the earlywood. This value is slightly lower than the value (0.22 g/g) found for Douglas-fir [77], although both loblolly pine and Douglas-fir tracheids have the same total lignin content. Although the S2 layer in normal wood is typically uniformly lignified, it may occasionally show concentric variations in lignification [78].

Lignin distribution in S1 is variously described as similar to the ML, intermediated between the ML and S2 layer, or similar to the S2 layer, depending on the species and technique used [6, 76]. Maurer and Fengel [79] described lignin distribution in the S1 region as being in two distinct parts, high in the outer and low in the inner regions for *Picea abies*. Donaldson [80] describes that the lignification of the S1 region in *P. radiata* is patchy and

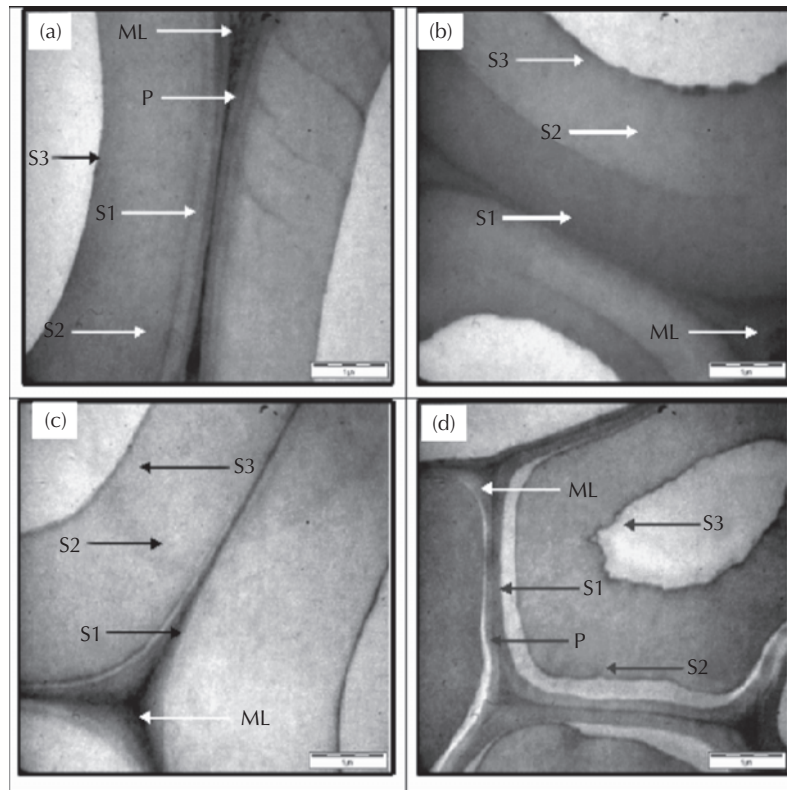
generally lower than that of the S2 layer, especially at the S1/S2 boundary.

The S3 layer has often been reported to be more lignified than the adjacent S2 layer. Quantitative studies indicate the values intermediate between the S2 and CML, but the general opinion is that the lignification state of the S3 layer is a variable feature. Lignin concentration in the S3 layer is similar to that in the S2 region in both Douglas fir and Larch [81]. Little information is available on species outside of the Pinaceae.

The lignin distribution between secondary wall and ML in hardwood is similar to that in softwood [82, 83]. As shown in Fig. 2.31, confocal images (530 nm) of *S. gordejecii* showed strongly lignified cell corner middle lamella (CCML) and weakly lignified CML and S2 layer [5]. Vessel secondary wall may be more highly lignified in some species. However, the secondary wall of hardwood fiber is often less lignified than the secondary wall in softwood tracheids. Fergus and Goring [84] found values for lignin concentration of 16–19% for the secondary wall of birchwood fibers, and 72–85% for the ML. Saka and Goring [70] studied lignin distribution in white birchwood fiber walls and very similar results were obtained. The average value for the S2 region was 16% (w/w), while for vessels the comparable value was 22%. The average value for the ML region was 72%.

### 2.3.2.2 Lignin Distribution in Grass

Although many chemical studies of grass lignin have been carried out, there appears to be little quantitative information on lignin distribution in grass species. The Br-L X-ray counts of the different brominated wheat straw cell



**FIGURE 2.25** Transverse section of agro fibers at higher magnification showing the multilayered structure. (a) Oil palm frond (OPF); (b) coconut (COIR); (c) banana stem (BS); (d) pineapple leaf (PALF),  $\times 17,000$  [40].

**TABLE 2.6** Polysaccharide Content in the Cell Wall Layers of Fibers and Teacheids [43]

Wood species	Polysaccharide	CML	S1	S2 outer part	S2 inner part + S3
<i>Betula verrucosa</i>	Galactan	16.9	1.2	0.7	0.0
	Cellulose	41.4	49.8	48.0	60.0
	Glucomannan	3.1	2.8	2.1	5.1
	Arabinan	13.4	1.9	1.5	0.6
	Glucuronoxytan	25.2	44.1	47.7	35.1
<i>Picea abies</i>	Galactan	16.4	8.0	0.0	0.0
	Cellulose	33.4	55.2	64.3	63.6
	Glucomannan	7.9	18.1	24.4	23.7
	Arabinan	29.3	1.1	0.8	0.0
	Glucuronoarabinoxylan	13.0	17.6	10.7	12.7
<i>Pinus Silvestris</i>	Galactan	20.1	1.6	1.6	3.2
	Cellulose	35.5	66.5	66.5	47.5
	Glucomannan	7.7	24.6	24.6	27.2
	Arabinan	29.4	0.0	0.0	2.4
	Glucuronoarabinoxylan	7.3	7.4	7.4	19.4

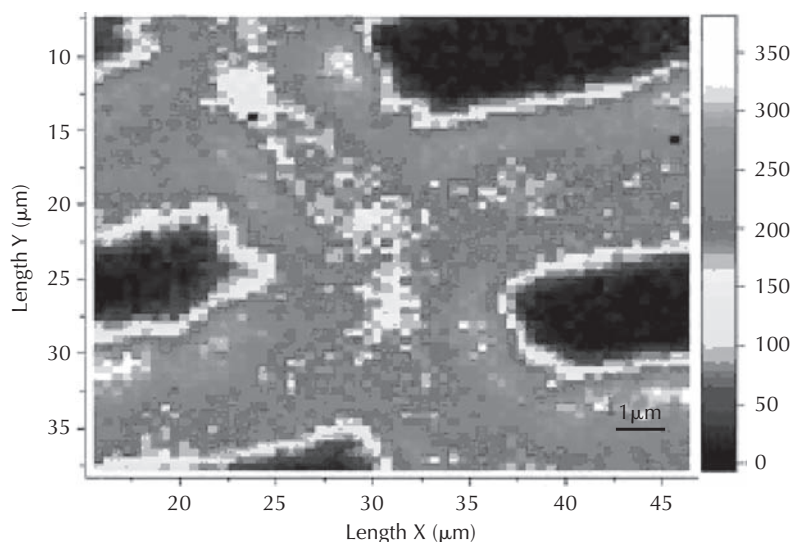


FIGURE 2.26 Raman images (false color) of cellulose spatial distribution in cell wall [4]. (see color plate)

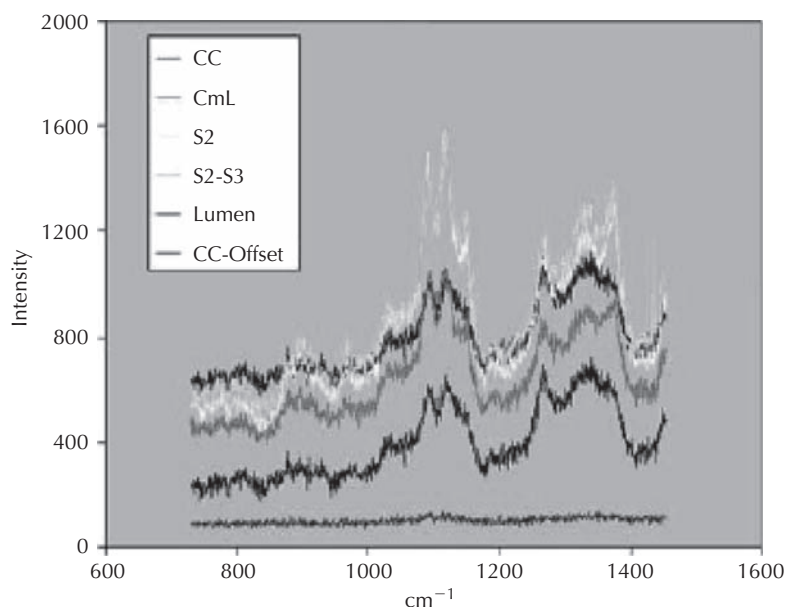


FIGURE 2.27 Typical spectra in cellulose frequency range (CC offset is same as CC but has been y offset by 400 intensity units for better visualization) [4]. (see color plate)

determined by the SEM-EDXA technique are shown in Table 2.10 [36]. The results indicated that in both fiber and nonfiber cells of wheat straw, the lignin concentration is the highest in the CC and the lowest in the secondary wall. In the morphological regions of all cells, the lignin concentration is the highest in parenchyma cells, followed by fibers, and is the lowest in epidermis cells. The latter is probably due to the higher content of silicon in epidermis cells [36]. Lignin concentrations in wheat straw, birch, and spruce are shown in Table 2.11. As can be seen, lignin concentration values are 15–16% (w/w) for the secondary wall, 34–41% for the ML, and 57–66% for the CC region. Lignin

concentrations in various morphological regions of wheat straw are similar to those in the corresponding regions of birch fiber. The lignin distribution in various morphological regions of wheat straw fiber is listed in Table 2.12. Because the volume fraction of the ML and the CC in wheat straw fiber is larger than that in wood fiber, the percentage of total lignin in the ML and the CC is greater for wheat straw fiber than for wood fiber [36].

Wheat straw was also examined by interference microscopy [69] and CLSM [85] to determine the lignin concentration in the ML and in the secondary wall. The results for lignin distribution, determined by interference



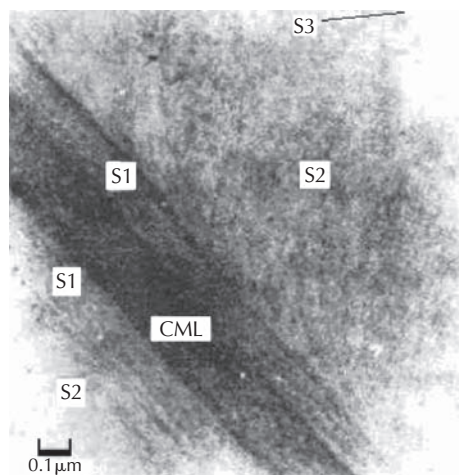


FIGURE 2.28 SEM image of cross section of spruce tracheids [58].

microscopy, showed that wheat fibers had a much lower level of lignification with a CCML concentration of only 31% varying from 28% to 35% and an S2 concentration of 9% varying from 7% to 9%. The results from CLSM also suggested reduced lignification of the ML region in the fiber or parenchyma cell walls of wheat straw, which was in agreement with the observations made using interference microscopy. A distinct S1 layer with slightly greater lignification than the adjacent S2 region was often observed. Fibers and parenchyma cells showed a similar level of lignification, while protoxylem and metaxylem vessel walls appeared to be more lignified than other cell types.

Lignin distribution in cell wall of reed was studied using SEM-EDXA in China Institute of pulp and paper [38]. Variations of absorption values in normal reed fiber at detective spot were found. The lignin distribution is the highest in CML, followed by S1 layer, and is the lowest in S2 layer. The results indicated that lignin concentration (absorption peak) in different layers is in agreement with the conclusions described by Saka et al. [76].

Recently, by staining with  $\text{KMnO}_4$ , it was revealed that lignin distribution in the ML of alfalfa (*Medicago sativa* L.) was irregular, as shown in Figs. 2.32 and 2.33 [86]. The lignification of the ML in the cells appears to be complete

or nearly complete. It is indicated that lignin distribution in the ML of the secondary xylem fibers in alfalfa stems is inhomogeneous. Lignin distribution in the ML is patchy, with a greater concentration in some areas than the others. It is also indicated that little or no lignin is present in some regions of the ML.

The lignin distribution in bamboo is different from the situation in wood. The high lignin content in the ML region was revealed. In general, the CCs have dense lignin content as shown by their corresponding contrast to ML. A dense network of lignin is characteristic of the narrow lamellar zones, with the intensity similar to that of the ML. However, the broad lamellar zones have a lower lignin concentration. Toward the lumen, the bordering region exhibits high lignin content [39].

As to different species of bamboo, the lignin distribution appears different. The distribution of lignin structural units in different anatomical regions was described, and lignification of the tropical bamboo species *Gigantochloa levis* (Fig. 2.34) was compared with that of the temperate bamboo species *Phyllostachys viridiglaucescens* [87]. Considerable differences were found in the structure of cell wall among fibers adjacent to the vascular tissue, fibers of free fiber strands, and parenchyma cells. In general, the S2 layer has a lamellar structure with increasing lignin content from the center towards the CML. *p*-coumaric and ferulic acids are more widely distributed in *Gigantochloa levis* (*G. levis*) and their content depends on the anatomical location. The early maturing fibers adjacent to the vascular tissue and at the outer culm wall reveal a maximum absorbance at 280 nm (guaiacyl peak), whereas the late maturing fibers display an absorbance at 310–320 nm. This is in contrast to *P. viridiglaucescens*, where the late maturing fibers also show a maximum peak at 280 nm. The CML show higher absorbance values and are richer in *p*-coumaric and ferulic acid esters in comparison to the S2 wall layers.

An observation has been made in four agro residues illustrated that thick-walled fiber in OPF, COIR, BS, and PALF were strongly lignified (Fig. 2.35). The ML showed a high level of lignification for all type of plant fibers. Phloem and parenchyma cells in OPF and BS fiber, which consists only of primary wall, were unlignified. Donaldson [85] also found that lignification in linseed flax was greater in xylem

TABLE 2.7 Hemicelluloses Content in the Cell Wall Layers of Three Species of Wood [58]

Wood species	CML	S1	Outer part of S2	Inner part of S2 + S3
<i>Betula verrucosa</i>	58.6	50.0	52.0	41.0
<i>Picea abies</i>	66.6	44.8	35.9	36.4
<i>Pinus Silvestris</i>	64.5	38.4	33.6	52.2

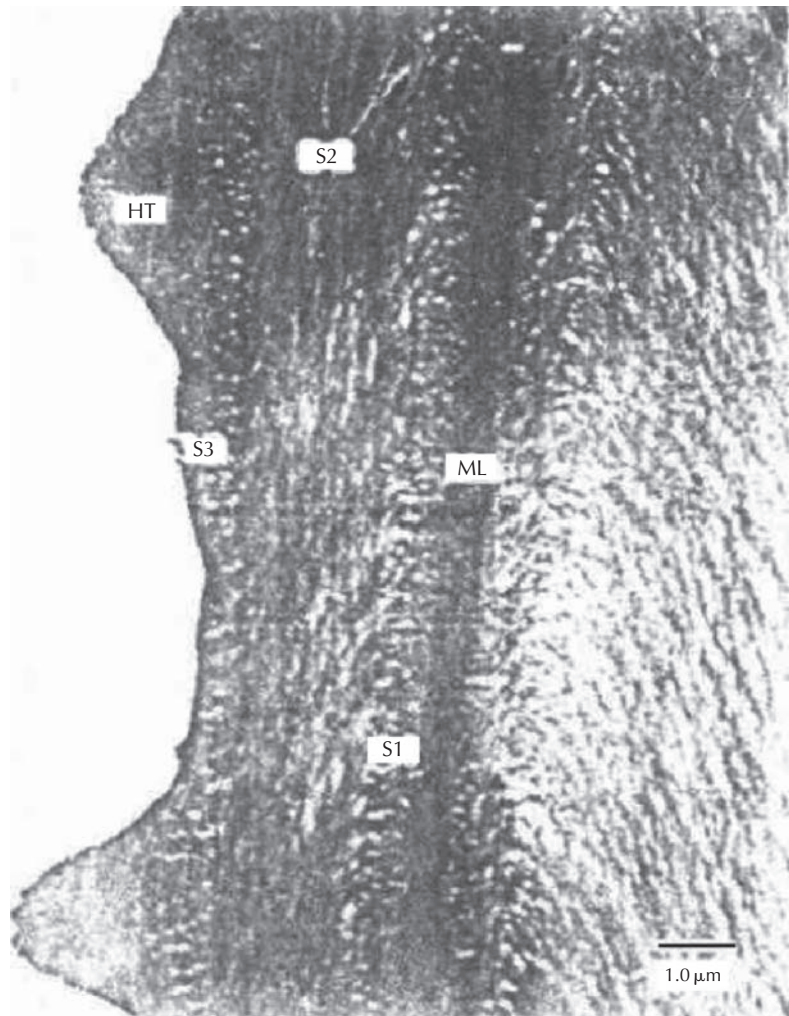


FIGURE 2.29 Lignin skeleton of *Pseudotsuga menziesii* cell wall [63].

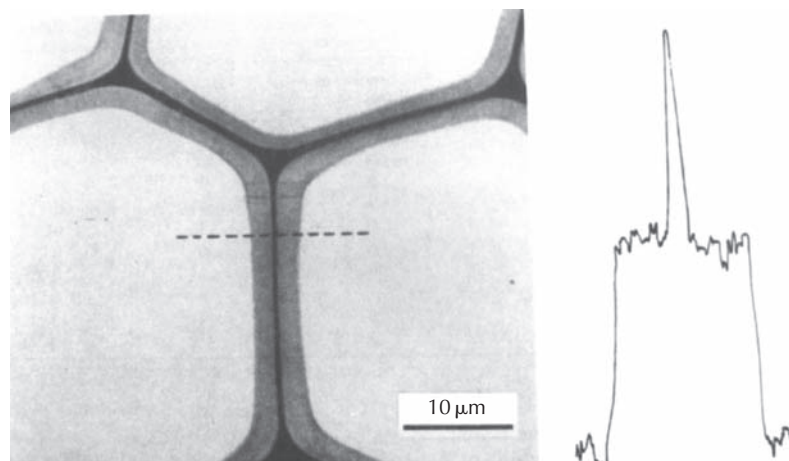


FIGURE 2.30 UV photomicrograph of earlywood tracheid walls in black spruce [66].

**TABLE 2.8** Lignin Distribution in Morphological Area of *Pseudotsuga menziesii* Xylem [66]

Morphological area		Tissue volume % of total	Average absorption ratio Ai	Lignin concentration (%)	Lignin concentration (g/g)
Secondary wall of tracheid	EW <sup>a</sup>	74.4	0.144	58.3	0.248
	LW <sup>b</sup>	89.7	0.125	77.9	0.228
CC	EW <sup>a</sup>	4.1	0.482	10.7	0.83
	LW <sup>b</sup>	1.9		6.2	0.88
Middle lamella	EW <sup>a</sup>	10.1	0.326	17.9	0.56
	LW <sup>b</sup>	4.6		10.3	0.60
Secondary wall of ray parenchyma	EW <sup>a</sup>	7.7	0.230	9.6	0.40
	LW <sup>b</sup>	2.6		4.1	
Secondary wall of ray tracheid	EW <sup>a</sup>	3.8	0.164	3.4	0.28
	LW <sup>b</sup>	1.3		1.5	
CML ( <i>i</i> 2,3)	EW <sup>a</sup>	14.2		28.7	
	LW <sup>b</sup>	6.5		16.6	
S ( <i>i</i> 1,4,5)	EW <sup>a</sup>	85.9		71.3	
	LW <sup>b</sup>	93.6		83.5	

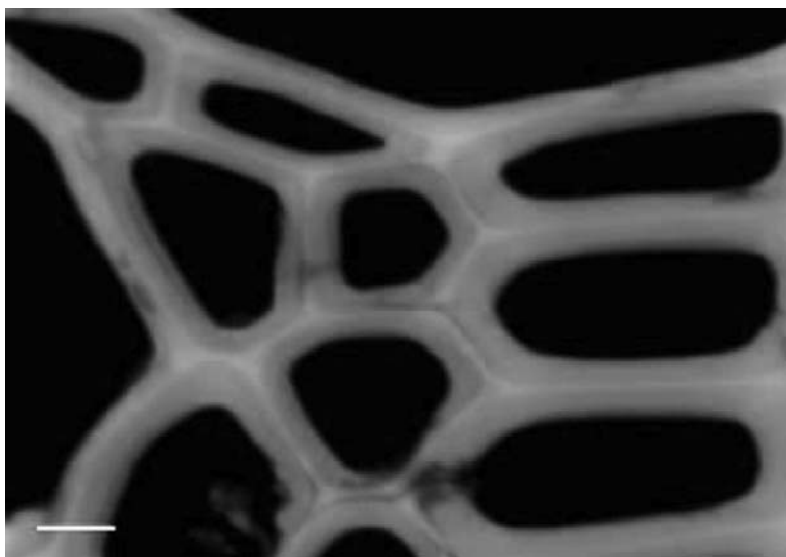
<sup>a</sup>EW: Earlywood.<sup>b</sup>LW: Latewood.**TABLE 2.9** The Distribution of Lignin in Loblolly Pine Tracheids as Determined by Bromination with SEM-EDXA [76]

Wood	Morphological region	Tissue volume (%)	Lignin % of total	Lignin concentration (g/g)
Earlywood	S1	13	12	0.25
	S2	60	44	0.20
	S3	9	9	0.28
	ML	12	21	0.49
	MLcc	6	14	0.64
Latewood	S1	6	6	0.23
	S2	80	63	0.18
	S3	5	6	0.25
	ML	6	14	0.51
	MLcc	3	11	0.78

fibers, compared to phloem fibers. In the OPF vascular bundle, fibers were more lignified than metaxylem vessels. Palisade and mesophyll cells, which were thin-walled parenchyma cells, also lignified weakly. These thin walls are important to absorb sunlight in an optimum manner to be used in the photosynthesis process.

## 2.4 CHEMICAL COMPOSITION

Straw consists mainly of three groups of organic compounds: cellulose, hemicelluloses, and lignin. Cellulose, hemicelluloses, and lignin account for more than 80% of the dry matter of the three more common British cereal



**FIGURE 2.31** Confocal scanning microscope image of fiber cell in normal wood of *S. gordejecii*. Scale bar = 7  $\mu$ m.

**TABLE 2.10** Br-L X-ray Counts of Different Kinds of Brominated Cells in Wheat Straw [36]

Cells	S	CML	CC
Thick wall fiber	1620	2156	2992
Thin wall fiber	1340	2004	3336
Parenchyma cell	2005	2599	
Epidermis cell	1022	1690	1743

straws such as oats, barley, and wheat [88]. In addition to these three main constituents, straw contains various other organic compounds including small amounts of protein, small quantities of waxes that protect the epidermis of the straw, sugars and salts, and insoluble ash including silica that is blunt to cutting machinery, reduces digestibility, interferes with pulping processes, and renders combustion

more difficult. Table 2.13 shows the chemical composition of agricultural residues (% dry matter) [89].

The chemical content of straw also varies appreciably according to the stage of maturity. The different fractions of straw vary in chemical composition (Tables 2.14 [90] and 2.15 [91]). Furthermore, it is known that the chemical composition of straws varies with soil type and fertilizer treatment. The analysis of straw will be affected by the proportion of the different fractions that exist in the sample. Table 2.14 shows that cellulose is higher in the internodes, and ash in which the main constituent is silica, insoluble ash, is high in leaf and leaf base. Lignin is high in the node cores.

It is well known that more than 100 different mono-saccharides are present in nature [92]. Luckily, 10 of those dominate quantitatively as building blocks of cell walls in higher plants. These are as follows: pentoses: arabinose and xylose; the hexoses: glucose, galactose, and mannose; the 6-deoxy-hexoses: rhamnose and fucose; and the hexuronic acids: galacturonic acid, glucuronic

**TABLE 2.11** Lignin Concentration in Various Morphological Regions of Straw and Wood Fiber [36]

Species	Fiber/Tracheid	Lignin concentration (g/g)		
		S	ML	CC
Wheat straw	Thick wall fiber	0.168	0.412	0.571
	Thin wall fiber	0.154	0.339	0.664
Birch	Fiber	0.16 0.19	0.34 0.40	0.72 0.85
Spruce	Earlywood tracheid	0.225	0.497	0.848
	Latewood tracheid	0.222	0.60	1.00



**TABLE 2.12** Distribution of Lignin in Different Morphological Regions [36]

Species	Fiber type	Lignin distribution (%)		
		S	ML	CC
Wheat straw	Thick wall fiber	67.5	18.0	14.5
	Thin wall fiber	57.5	20.3	22.2
Birch	Fiber	77.5	11.3	11.2
Spruce	Earlywood tracheid	72.1	15.8	12.1
	Latewood tracheid	81.7	9.7	8.6

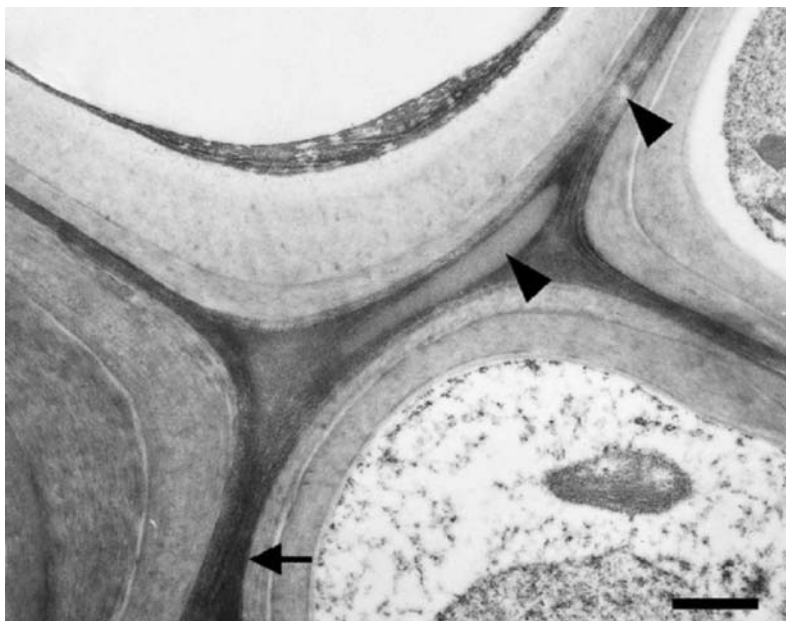
acid, and 4-*O*-methylglucuronic acid (Fig. 2.36). These 10 different monosaccharides can theoretically form an astronomical number of polysaccharides, which results in the division of carbohydrate polymers into classes according to their physical, chemical, or biological properties [93].

### 2.4.1 Cellulose

Cellulose is the most abundant organic polymer on earth, being found in all land plants and even in many aquatic species such as algae. Cellulose is distributed in all plants from highly developed trees to primitive organisms such as sea-weeds, flagellates, and bacteria. Cellulose can even found in the animal kingdom [94]. Cellulose is a linear homopolymer of  $\beta$ -1,4-linked D-anhydroglucopyranosyl

units, which occurs in nature largely in a crystalline form, and organized as fibrils [91]. These cellulose fibers constitute the skeleton of both the primary and the secondary cell wall. Glucose units per molecule (degree of polymerization) range from 15 to as high as 10 14,000 [95], or up to 15,000 [96], and molecules are arranged in antiparallel fashion i.e. with adjacent chains running in opposite directions. These cellulose molecules are aggregated into long slender bundles called microfibrils (Fig. 2.37) [97]. Within the microfibrils, linear molecules are bound laterally by hydrogen bonds [98] and are associated in regions that contain highly orientated molecules that are referred to as crystalline regions and those of lesser order called paracrystalline or amorphous regions [95]. Various arrangements have been proposed for the crystalline and amorphous regions (Fig. 2.38) [95].

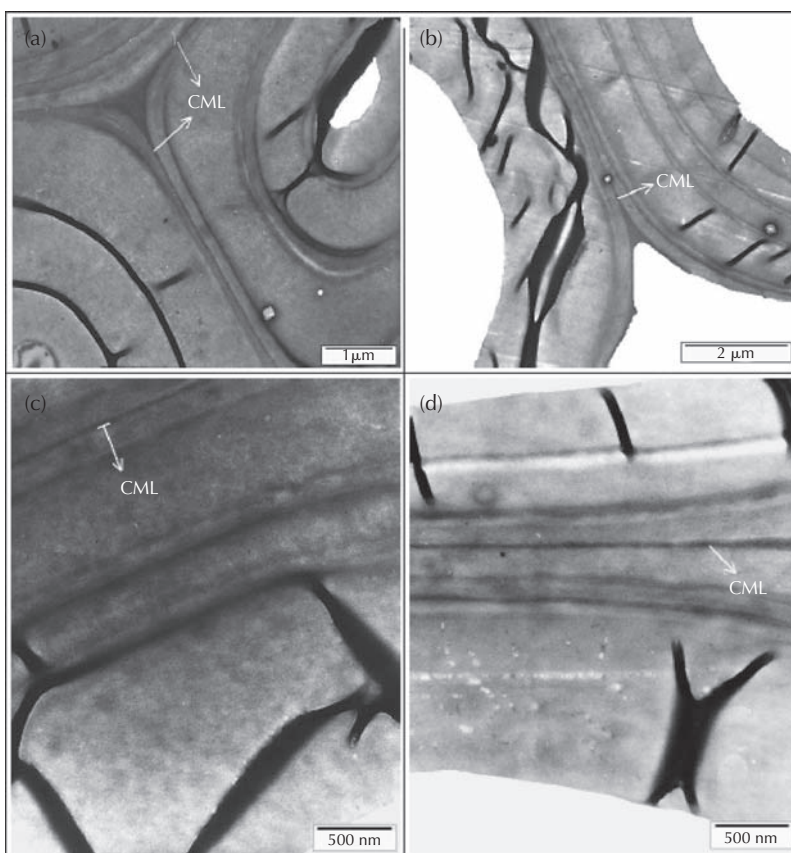
The hydrogen bonding is very strong and is only disrupted by very strongly aprotic solvents and also strong acids and alkalis. A single cellulose chain is not restricted to one fibril: noncrystalline regions are present where one chain goes from one fibril to another producing an even more varied network [88]. The conformation of the cellulose molecule in contrast to the chemically related starch polymers, having  $\alpha$ -1,4-linkages, favors the formation of such bonds, both internal and external. At the molecular level, the crystalline cellulose core of cell-wall microfibrils [99] is highly resistant to chemical and biological hydrolysis because of its structure, in which chains of celloextrins are precisely arranged. The chair conformation of the glucose residues in cellulose forces the hydroxyl groups into radial (equatorial) orientation and the



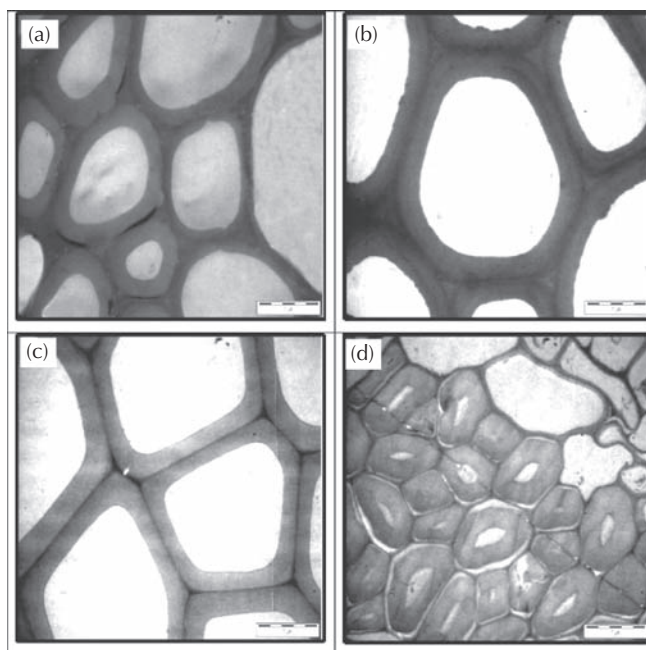
**FIGURE 2.32** Transverse section through secondary xylem fibers of alfalfa undergoing secondary wall thickening and lignification. The density of the ML is highly variable with regions of high (arrow) and poor (arrow heads) electron density, and correspondingly high and low in lignin concentration. Scale bar = 2  $\mu$ m [86].



**FIGURE 2.33** Transverse section of alfalfa through a secondary xylem region containing mature fibers [86]. Note the presence of electron lucent regions in the CC and intercorner ML (arrows). Scale bar = 1  $\mu\text{m}$ .



**FIGURE 2.34** (a) Cell wall layers of fibers adjacent to the vascular tissue of a 21-month-old culm of *G. levis*. (b) Cell wall layers of fibers of free fiber cap of an 8-month-old culm of *G. levis*. (c) Detail of cell wall layers of fibers adjacent to the vascular tissue of an 8-month-old culm of *G. levis*. (d) Detail of cell wall layers of fibers of free fiber cap of an 8-month-old culm of *G. levis* [87].



**FIGURE 2.35** Transmission electron micrograph of ultrathin section of agro fibers after stained with uranyl acetate and lead citrate at low magnification,  $\times 3400$ . (a) OPF; (b) COIR; (c) BS; (d) PALF [40].

**TABLE 2.13** Chemical Composition of Agricultural Residues (% Dry Matter) [89]

Species	Water-solubles	Cellulose	Hemicelluloses	Lignin	Wax	Ash
Wheat straw	4.7	38.6	32.6	14.1	1.7	5.9
Rice straw	6.1	36.5	27.7	12.3	3.8	13.3
Rye straw	4.1	37.9	32.8	17.6	2.0	3.0
Barley straw	6.8	34.8	27.9	14.6	1.9	5.7
Oat straw	4.6	38.5	31.7	16.8	2.2	6.1
Rape straw		37.6	31.4	21.3	3.8	6.0
Maize stems	5.6	38.5	28.0	15.0	3.6	4.2
Corn cobs	4.2	43.2	31.8	14.6	3.9	2.2
Esparto	6.1	35.8	28.7	17.8	3.4	6.5
Sugar beet pulp	(pectin 27.1) 5.9	18.4	14.8	5.9	1.4	3.7
Bagasse	4.0	39.2	28.7	19.4	1.6	5.1
Rye grass	8.5	37.6	32.2	8.2	4.4	4.5
Oil palm fiber	5.0	40.2	32.1	18.7	0.5	3.4
Abaca fiber	3.7	60.4	20.8	12.4	0.8	2.5

aliphatic hydrogen atoms into axial positions. As a result, there is a strong interchain hydrogen bonding between adjacent chains in a cellulose sheet and weaker hydrophobic interactions between cellulose sheets. The hydrophobic face of cellulose sheets makes crystalline cellulose resistant to

acid hydrolysis because it contributes to the formation of a dense layer of water near the hydrated cellulose surface [100]. The accessibility of cellulose to hydrolysis can be enhanced by milling treatments to increase the surface area, and steaming or treating with swelling agents, such as

**TABLE 2.14** The Chemical Components of Different Parts of Wheat Straw Determined by Gravimetric Analysis [90]

	Percentage of dry weight				Least significant difference ( $p = 0.05$ )
	Leaf	Internode	Leaf base	Node core	
Hot water soluble	14.6	7.2	18.9	13.2	1.8
Lignin	15.3	14.2	14.1	16.7	1.2
Hemicelluloses	32.4	33.8	34.2	32.7	NS
Cellulose	37.7	44.8	32.7	37.5	3.6
Total N	0.3	0.3	0.4	0.5	NS
Ash	11.5	4.7	12.4	6.3	1.9

NS, not significant.

**TABLE 2.15** Fractionation and Chemical Composition of Straw (% of Dry Weight) [91]

Cultivar	Ash	Protein ( $N \times 6.25$ )	Extractives (80% EtOH)	Hemicelluloses	Cellulose	Lignin (Klason)
Winter wheat						
Holme (locality 1)	8.0	3.0	5.2	31	35	21
Holme (locality 2)	10.7	5.1	5.1	33	37	21
Barleys						
Ida	3.0	3.1	6.0	33	40	16
Lajsa	6.5	3.3	8.1	31	36	19
Tellus	5.5	3.6	8.1	31	35	18
Oats						
Titus	6.0	4.5	7.4	31	33	18
Sang	6.4	2.8	6.4	29	38	19
Pol	5.8	1.9	6.8	32	36	19
Selma	3.9	2.7	7.1	32	36	19

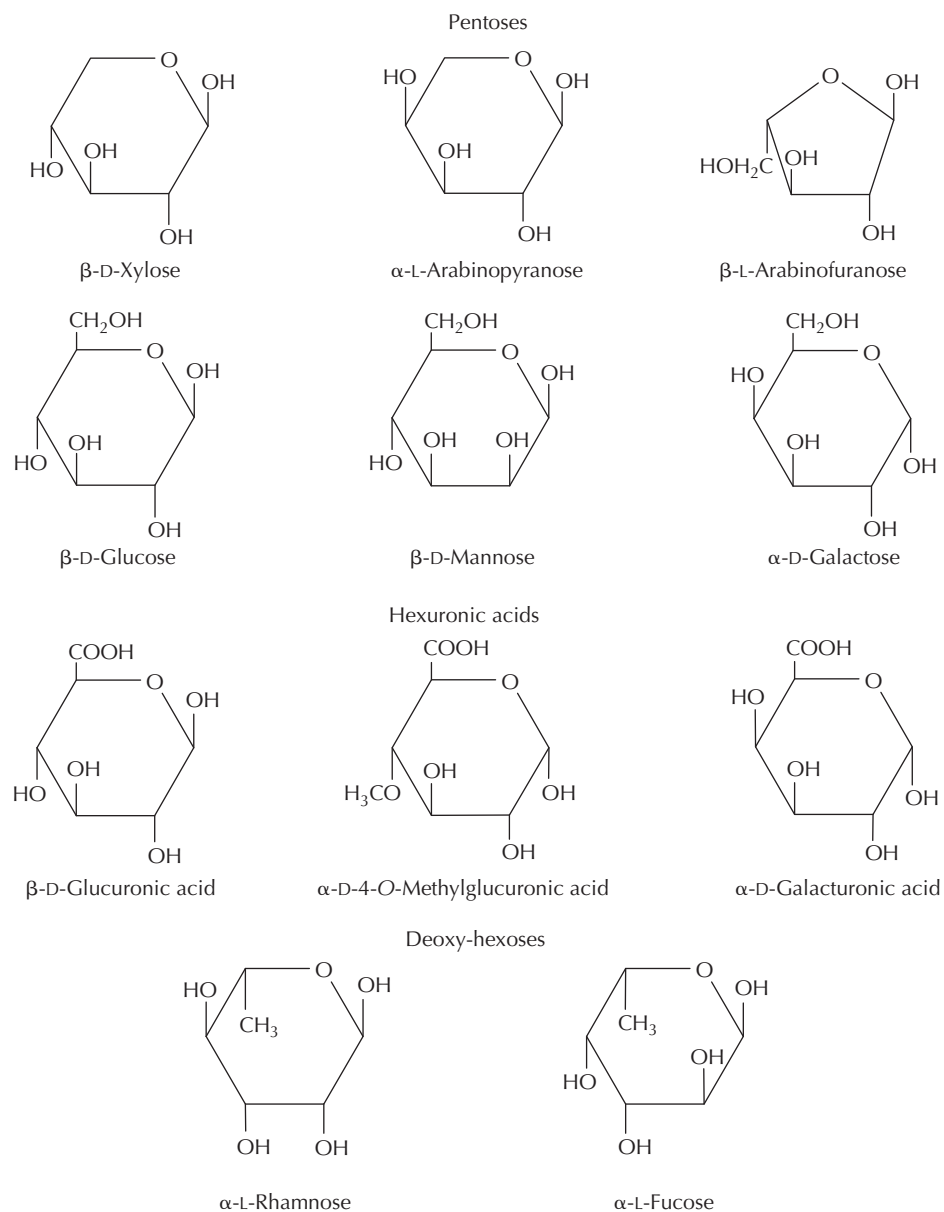
alkali, which can make the cellulose less crystalline and/or less hindered by associated components, such as lignin or silica [91]. In any isolation method, cellulose cannot be obtained in a pure state, but only as a more or less crude preparation which is generally called  $\alpha$ -cellulose, that is insoluble in a strong sodium hydroxide solution. The portion that is soluble in the alkaline medium but precipitable from the neutralized solution is called  $\beta$ -cellulose.  $\gamma$ -cellulose is the name for the portion that remains soluble even in the neutralized solution.

## 2.4.2 Hemicelluloses

Hemicelluloses rank second to cellulose in abundance in cereal straws, comprising roughly one-fourth to one-third of

most plant materials, and this amount will vary according to the particular plant species (Table 2.13) [89]. Unlike cellulose, which is a unique molecule differing only in degree of polymerization and crystallinity, the hemicelluloses are noncrystalline heteropolysaccharides and classically defined as the alkali soluble material after removal of the pectic substances. This definition of hemicelluloses is very generic but is accepted at present [101]. Hemicelluloses, however, are the most complex components in the cell wall of cereal straws and grasses. They form hydrogen bonds with cellulose, covalent bonds (mainly  $\alpha$ -benzyl ether linkages) with lignin, and ester linkages with acetyl units and hydroxycinnamic acids. In addition, hemicelluloses are formed through biosynthetic routes different to the glucose-UDP route of cellulose (a homopolysaccharide). They are





**FIGURE 2.36** Main cell wall monosacchride components.

branched polymers of low molecular weight with a degree of polymerization of 80–200. Their general formulae are  $(C_5H_8O_4)_n$  and  $(C_6H_{10}O_5)_n$  and they are called as pentosans and hexosans, respectively [102]. Hemicelluloses consist of various different sugar units that are arranged in different proportion and with different substituents [103–108]. The principle sugars are D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid, 4-O-methyl-D-glucuronic acid, D-galacturonic acid, and to a lesser extent, L-rhamnose, L-fucose, and various O-methylated neutral sugars. A number of hemicelluloses are neutral molecules, but by far the most abundant have a backbone of (1→4)-linked  $\beta$ -D-xylopyranosyl units. The chain may be linear but

is often branched and usually has other glycosidically bound sugar units.

Hemicelluloses of cereal straw from the Gramineae family have a backbone of (1→4)-linked  $\beta$ -D-xylopyranosyl units. Some xylan chains have D-glucopyranosyluronic acid units attached, but the most important acidic hemicelluloses are O-acetyl-4-O-methyl-D-glucuronoxylans and L-arabino (4-O-methyl-D-glucurono)xylans. Xylans from grasses and cereal straws contain smaller proportions of uronic acids, but they are more highly branched and contain large proportions of L-arabinofuranosyl units. In general, arabinofuranosyl units are attached to some C-3 position of the main xylan chain and glucuronic acid and/or its 4-O-methyl

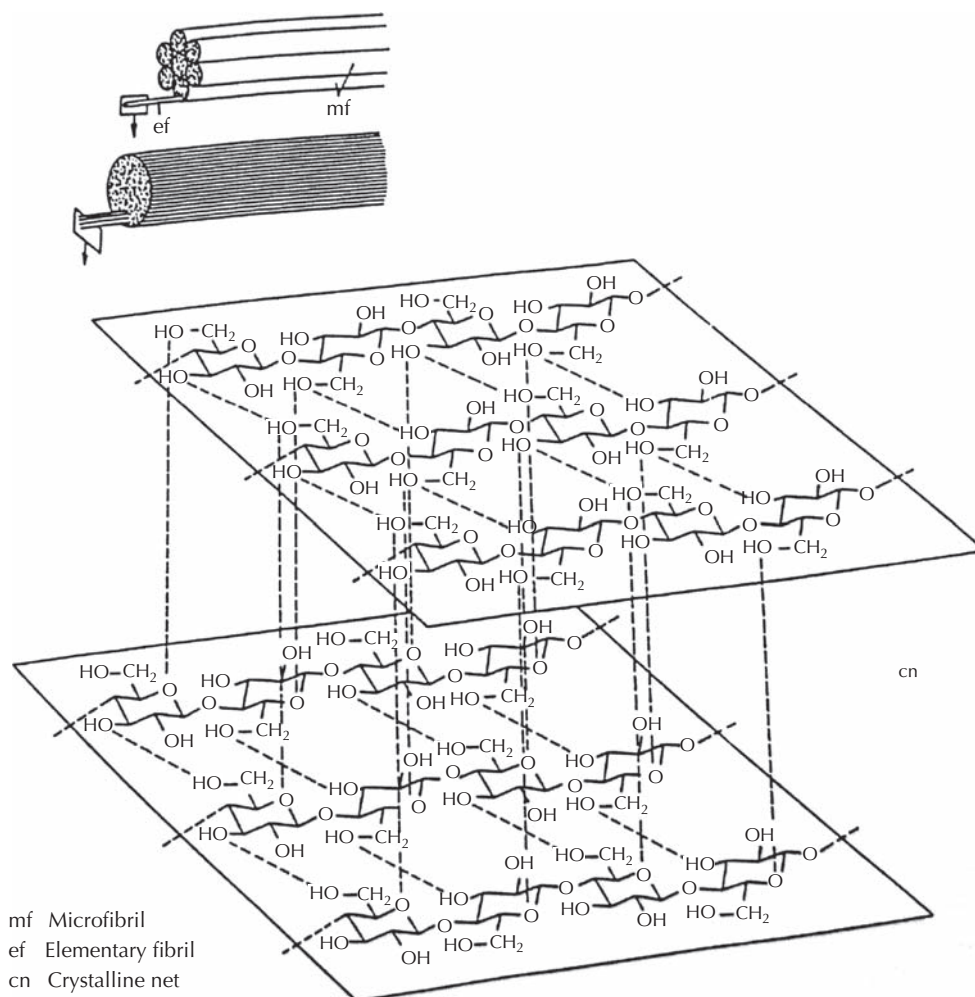


FIGURE 2.37 Molecular and fibrillar structure of cellulose [97].

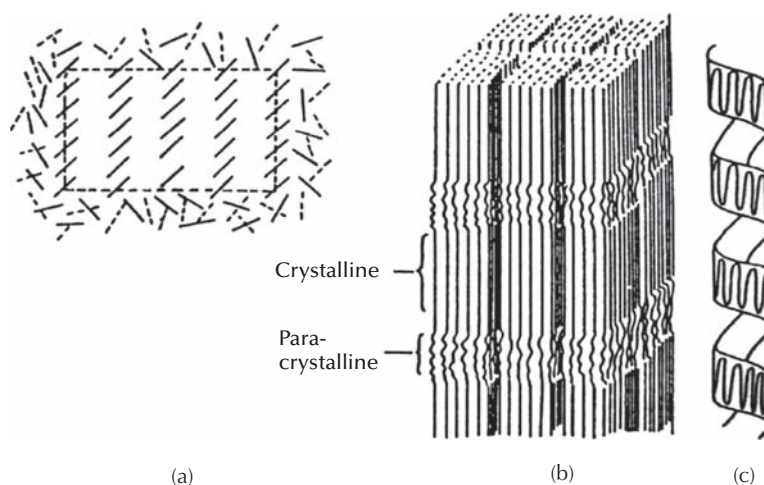


FIGURE 2.38 Concepts of the structure of cellulose microfibrils (a) Microfibril consists of a rectangular crystalline core surrounded by a paracrystalline cortex (solid lines represent planes of glucose residues; broken lines, the orientation of hemicellulose molecules). (b) Linear microfibrils containing 15-40 cellulose molecules segmented into crystalline and paracrystalline regions. (c) Cellulose molecule first folded into a flat ribbon and then wound in a right helix [95].

ether linked to some xylose units. The latter are probably mainly linked to the C-2 position [109]. The fact that the arabinose units generally seem to be furanosidically linked as terminal groups makes them particularly sensitive towards acid hydrolysis [91, 101]. The arabinose and uronic acid xylans of these hemicelluloses vary considerably from the cereal grain arabinoxylans that have an arabinose: xylose ratio of about 1 to esparto xylan which has no arabinose at all. During botanical aging, the percentage of side chains on xylans decreases markedly; hence, straw xylans [109] are found to have relatively lower arabinose content as compared to the xylans from cereal grains [88]. In the primary wall, the 4-*O*-methyl-D-glucuronic side chains seem to be absent [110]. However, oligomeric side chains containing other glycosyl residues, e.g. galactose, are also found. An acid galactoarabinoxylan has been isolated from the cell wall of Gramineae by Buchala et al. [111]. A significant portion of the xylose in cereal straw cell walls was found to be acetylated, mainly on C-2 but also on C-3 [107]. Bacon et al. [112] mentioned that the cell walls of Gramineae plants account for 1–2% of the acetyl groups. These acetylated hemicelluloses are soluble in water and in solvents such as dimethyl sulfoxide, formamide, and *N,N*-dimethylformamide. Furthermore, cell walls of cereal straws also contain 1–2% phenolic acids, which are esterified to hemicelluloses. *p*-Coumaryl and ferulyl groups are attached to the xylan through the arabinose residues at C-5 position [113]. It is estimated that 1 of 121 pentose residues is ferulylated and 1 of 243 is *p*-coumarylated in barley straw [114].

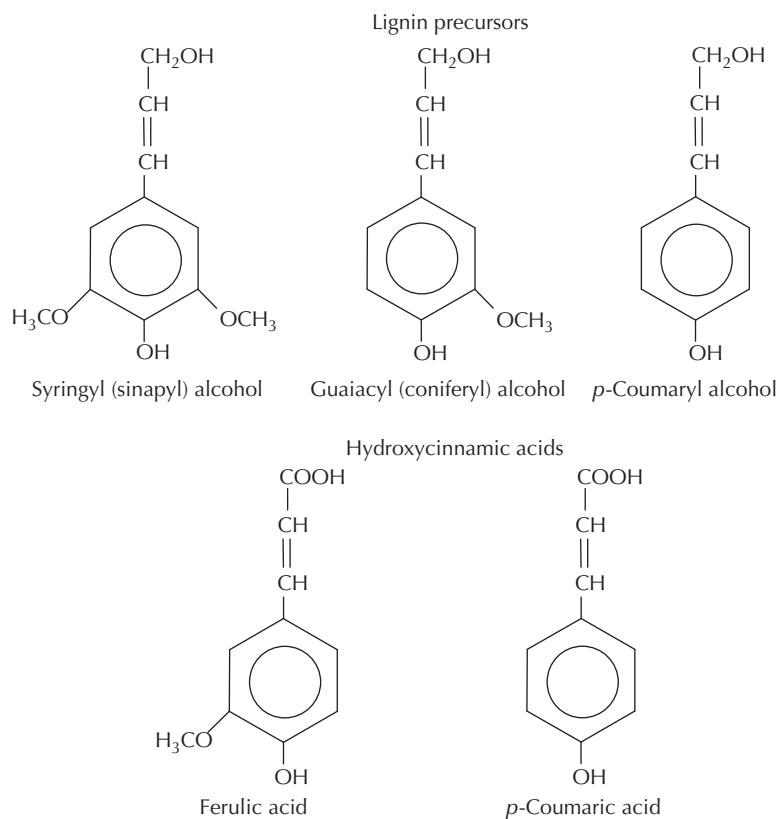
In addition to 4-*O*-methylglucuronarabinoxylan, other important hemicelluloses are xyloglucans and glucomannans. The xyloglucans in primary cell walls of monocotyledonous plants account for only about 2% of the cell wall [115]. Xyloglucans have a backbone of (1→4)- $\beta$ -linked glucose residues with (1→6)- $\alpha$ -linked xylose, galactose, and fucose residues in various proportions as side chains [116]. The xyloglucan attaches tightly to cellulose microfibrils and it is suggested that hydrogen bonding between xylose containing polysaccharides on the microfibrils is responsible for the bundling into cellulose fibers [117]. Glucomannan is essentially a linear polysaccharide composed of both (1→4)- $\beta$ -linked D-mannose and D-glucose residues. Some terminal D-galactosyl residues are  $\alpha$ -linked to C-6 of the residues of the main chain [118]. Noncellulosic  $\beta$ -glucans have been found to be present in most cereal endosperm cell walls, such as the stems of barley and oats [119, 120] or in the primary walls of vegetative parts of some monocots [121]. These polymers are distinguished from the cellulose by the presence of both  $\beta$ -(1→3) and  $\beta$ -(1→4)-linked D-glucosyl residues and by lower molecular weight [118]. A structure of wheat straw hemicelluloses was given by Sun et al. [122], which will be given in Chapter 4.

### 2.4.3 Lignin

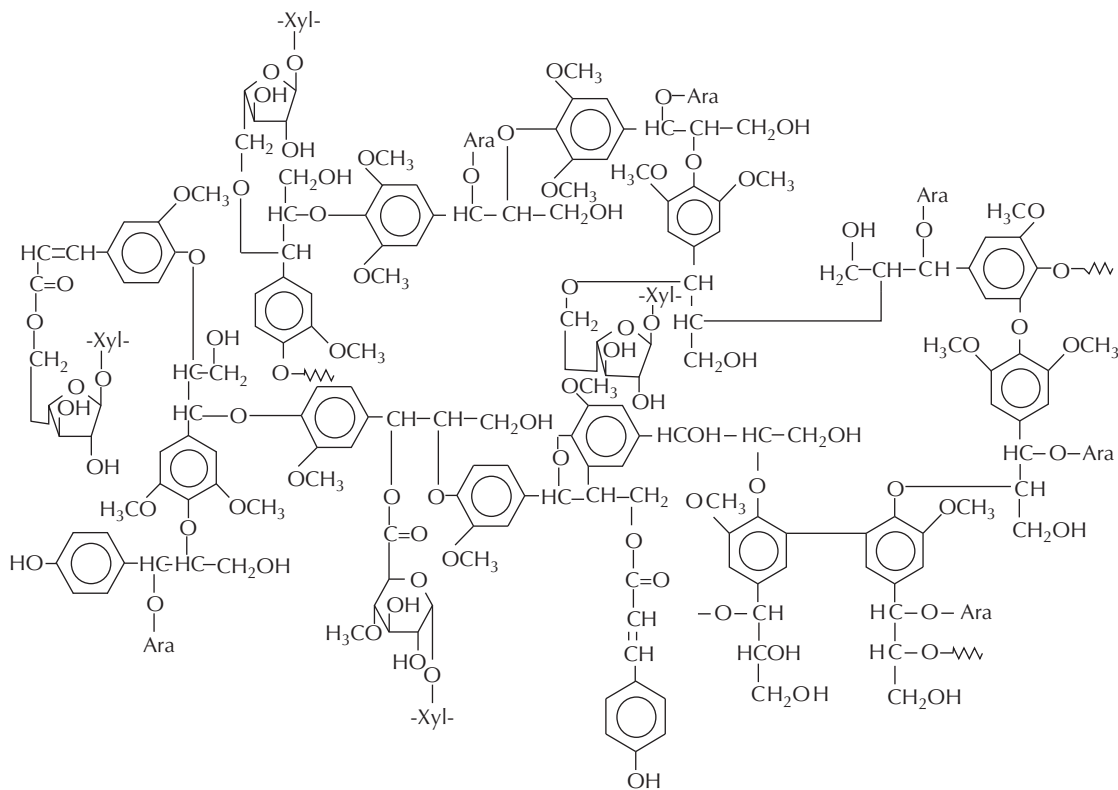
Lignin is one of the most abundant organic materials and renewable resources on earth. Apart from the polysaccharides, lignin, a family of branched noncarbohydrate polymers, is a main component of straw (Table 2.13) [89]. According to the widely accepted concept, lignin may be defined as an amorphous, polyphenolic material arising from an enzyme-mediated dehydrogenative polymerization of three phenylpropanoid monomers, coniferyl, sinapyl, and *p*-coumaryl alcohols (Fig. 2.39). This definition, however, has long been recognized as too narrow [123]. Many plants have lignins containing significant levels of other unusual components, and it is likely that no plant contains lignins that are solely derived from the three “primary” precursors. For example, evidence from mutants and genetic variants where aldehydes accumulate strongly supports the view that aldehydes are incorporated as precursors, because, in these variants, more aldehydes are found in the lignin [124–128]. For this reason, lignin is viewed not as a constitutionally defined compound, but as a composite of physically and chemically heterogeneous materials whose structure may be represented by models such as those proposed for wheat straw (Figs. 2.40) [129]. This model should not be regarded as depicting the structural formulas for lignin in the usual sense, but as vehicles for illustrating the types and linkage modes of the constituent structural elements and the proportions in which they are believed to occur in lignin.

Lignin deposition is probably initiated in the CCs when the surface enlargement of the cell is completed and just before the secondary wall starts thickening. The lignification proceeds in the ML and the primary wall. The lignification of the secondary wall proceeds slowly in the first stage but becomes faster after the thickening has been completed [118, 130]. The hydrophobic nature and the low content of hydrolyzable bonds render lignin very durable, and thus, lignin can serve as a protection against mechanical as well as microbial injury. Even though lignin is present in most nonendospermic tissues of straw or grasses, it is either absent from, or present in low proportion in, young cells and root tissues [131]. Lignin is generally distributed with hemicelluloses in the spaces of intercellulose microfibrils in primary and secondary walls, and in ML as a cementing component to connect cells and harden the cell walls of xylem tissues [132]. About 60–80% of the total lignin is located within the secondary wall [84, 133].

The major interunit linkage is an aryl aryl ether type. In addition to the phenylpropanoid units, smaller amount of C<sub>6</sub>-C<sub>1</sub> units are found in some lignin samples, especially *p*-hydroxybenzoic acid units, which may be linked via ester and ether bonds to the rest of the lignin molecule [134]. Besides some 20 different types of bonds present within the lignin itself, lignin seems to be particularly associated with



**FIGURE 2.39** The basic phenylpropanoid units of lignin (upper) and the most common hydroxycinnamic acids found in cereal straw cell walls (lower).



**FIGURE 2.40** Structure model of wheat straw lignin [129].



the hemicellulosic polysaccharides [91, 135, 136]. Some covalent linkages have also been proposed between lignin and other structural polymers of the cell wall, e.g., proteins [134]. In contrast to all other organic building blocks of the cell wall, lignin has no optical activity. Plants use lignin to (1) add strength and structure to their cellular composites; (2) control fluid flow; (3) protect against attack by micro-organisms; (4) act as an antioxidant, a UV absorber, and possibly a flame retardant; and (5) store energy [137]. Owing to its reticulation, lignin in situ is usually insoluble in all solvents, unless it is degraded by physical or chemical treatments.

Lignins have been generally classified into three major groups based on the chemical structure of their monomer units: softwood lignin, hardwood lignin, and grass lignin [132]. Depending on its composition of guaiacyl (G), syringyl (S), and *p*-hydroxyphenylpropane (H) units, cereal straw lignin or grass lignin has been justified as GSH-lignin (Gramineae lignin from grasses), which are known to be different from those of softwood (G-lignin) or hardwood (GS-lignin) and compression wood (GH-lignin) lignin. Furthermore, hydroxycinnamic acids, mainly *p*-coumaric, and ferulic acids have been investigated as cross-links between lignin and polysaccharides [138–141]. The other group in straw lignin was found to be acetyl residues. There are not many estimates of the acetyl content of straws but by analogy with woods and some grass residues. In barley straw, the values of 1–2% dry matter are probably accurate [88]. The complex nature of straw lignin and the difficulty of isolation of relatively pure lignin from grasses or cereal straw have made the progress in obtaining structural information on grass or straw lignin slower than the progress on wood lignin.

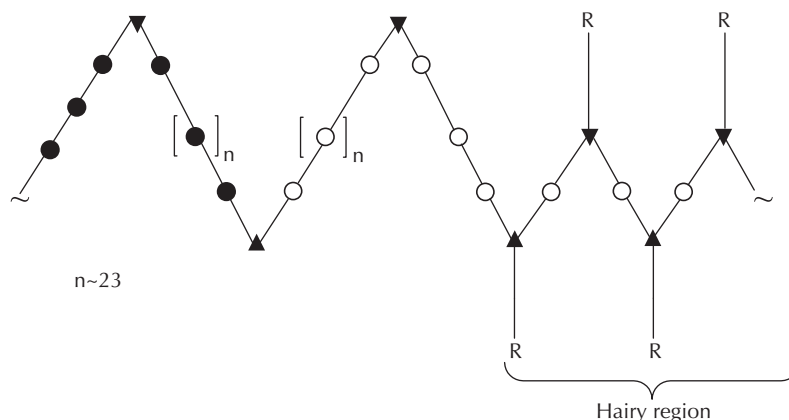
A major problem in native lignin structure elucidation has been in trying to isolate as much of the lignin as possible while minimizing the extent of chemical modification. The studies on lignin can be divided into two clearly separated fields: qualitative and quantitative studies. Qualitative analysis of lignin generally has the aim of defining the H/S/G ratio and the nature of the interunit bonds with destructive methods such as acidolysis [142], hydrogenolysis [143], nitrobenzene oxidation [144], cupric (II) oxidation [145], permanganate oxidation [146], ozonation [147], thioacidolysis [148], and derivatization followed by reductive cleavage (DFRC), which was proposed by Lu and Ralph [149]. All these destructive methods could provide information regarding the structure of lignin through the generation of low-molecular weight compounds. Through careful analyses of these compounds, a detailed picture of the original lignin can emerge. Quantitative analysis is based on gravimetry or UV-absorption [150] either to estimate lignin as an insoluble residue after strong sulfuric acid treatment, Klason lignin [151], or to oxidize the lignin away from a holocellulose

preparation, acidic chlorite lignin or permanganate lignin [152]. Spectroscopic techniques, such as infrared (IR) and  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy are complementary to the aforementioned degradative procedures because they provide information on the whole structure of the polymer and avoid the possibility of degradation artifacts [138].

#### 2.4.4 Pectic Substances

The pectic substances are found universally in the primary cell walls and intercellular layers in land plants. They are most abundant in soft tissues such as the rinds of citrus fruit (about 30%), sugar beet pulp (25%), and apples (15%) [110], but they are present in only small proportions in woody tissues (about 1%). The term of pectins or pectic substances are associated with acidic polysaccharides consisting of a backbone of mainly (1→4)- $\alpha$ -bound D-galacturonic acid residues interrupted by the insertion of (1→2)- $\alpha$ -linked L-rhamnose residues [93]. Other constituent sugars such as L-arabinose, D-galactose, D-xylose, L-fucose, and traces of 2-*O*-methyl-D-xylose and 2-*O*-methyl-L-fucose are attached in the side chains. Arabinose and galactose residues are the most common side chains found, whereas rarer monosaccharide residues such as the branched chain sugars apiose and acetic acid (3-C-carboxy-5-deoxy-L-xylose) have been found in small but significant amounts [153].

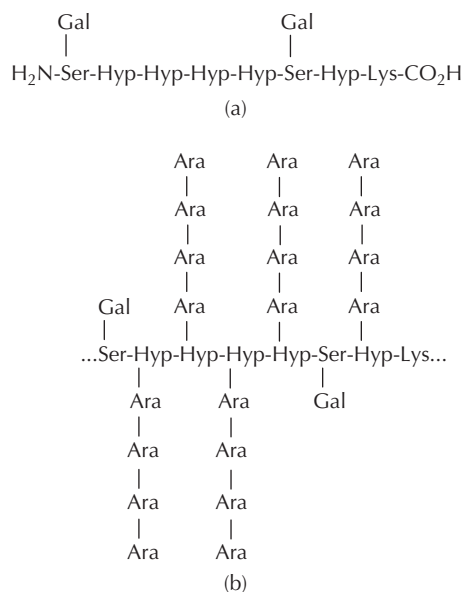
Polysaccharides in which a proportion of the D-galacturonic acid residues are present as methyl ester are designated pectinic acids, and those devoid of ester groups as pectic acids. In pectic substances, a high proportion of the D-galacturonic acid residues are esterified as methyl ester, and those which are most readily extracted with water or dilute acid possess considerable gelling power and are widely used for the gelation of fruit juices to form jellies. The pectic substances in the cell wall, in contrast to those in the intercellular layer, tend to occur with a proportion of the acid groups as salts (most frequently of calcium) and are extracted with ammonium oxalate or reagents such as sodium hexametaphosphate or ethylenediamine tetraacetate, which complex with divalent metal ions [110]. The pectic substances are block polymers with sections of unbranched backbone structure of rhamnogalacturonans and “hairy regions” with frequent side chains linked at C-4 of the rhamnose residues (Fig. 2.41) [93]. The methyl-esterified pectic substances can produce acid type gels, while the nonmethyl-esterified pectins are capable of forming gels with  $\text{Ca}^{2+}$  ions [154]. The polygalacturonic acid chain can also be acetylated to various extents, and other substituents that have been found are ferulic acid [155] and the amino acid derivative isodityrosine [93, 156].



**FIGURE 2.41** Schematic drawing of pectin with blocks of methyl esterified (○) and unesterified (●) galacturonic acid residues (▼) rhamnose residues and a branched “hairy region” indicated (R side chains rich in arabinose and galactose residues) [93].

### 2.4.5 Proteins

The plant cell wall is central to many developmental processes and in addition forms the bulk of plant biomass. Cell surface polysaccharides, proteoglycans, and glycoproteins are likely to have diverse roles in relation to the formation of plant cell wall architecture and the integration of the cell wall with the cell interior [157]. Glycoproteins are invariably found in primary cell walls. A particular protein (extensin), of which the uncommon amino acid hydroxyproline constitutes up to 20% of the amino acid content, is found in many dicots [158] as well as monocots [159]. This protein makes up to 2–10% of the primary cell wall, and most of the hydroxyproline residues are glycosylated by a tri- or tetra-arabinoside [158, 160]. Many of the serine residues are also glycosylated but with galactose residues. Hydroxyproline-rich glycoproteins containing arabinose residues have been isolated from monocotyledonous plants [161]. Cell walls obtained from tomato suspension cultures were treated at pH 1 for 1 h at 100°C to remove arabinose oligosaccharide substituents from the hydroxyproline residues of extensin. Tryptic attack of these acid-stripped walls yielded glycopeptides containing galactose [162]. The authors also found that most of the cell-wall-bound hydroxyproline is *O*-substituted by tetraarabinose (Fig. 2.42). A galactosaminoglycan moiety has been obtained from an antitumor polysaccharide fraction isolated from *Cordyceps ophioglossoides* culture [163]. Knox et al. [157] also mentioned the complex of arabinogalactan-proteins isolated from plant cell walls. A derivative of the amino acid tyrosine, isodityrosine (IDT), which can serve as a cross-linking agent between different cell wall proteins, has been identified by Fry [156]. Cooper and Vamer [164] indicated that IDT is produced from tyrosine in glycoproteins during insolubilization. This would imply that extension is immobilized by the dimerization of tyrosine to IDT when deposited in the primary wall [93]. Of amino acids, glutamic acid, proline, lysine, and phenylalanine prevailed in the



**FIGURE 2.42** Structures of protein hemicelluloses: (a) galactan proteins; (b) galactoarabinan proteins [162].

wheat straw analyzed [165]. This overall composition and proportion of individual amino acids in wheat straw is typical for proteins occurring in plants.

### 2.4.6 Cutins, Suberins, Waxes and Other Extracts

The surface of plants is important in protecting the plant from the effects of water loss, from the effects of high and low temperatures, and from the effects of insect predation. On the aerial parts of the plant, the cuticle is made up of cutin and waxes, whereas the subterranean parts are protected by suberin also associated with waxes [166]. The first barrier to insect attack is the epicuticular wax layer. Cutin and suberin are lipophilic polymers. Cutin is

In contrast to cutin, suberin does not constitute a morphologic unit but is embedded in the epidermal wall of

underground parts of the plant [168]. Suberin is, like cutin, a copolymer of aliphatic and aromatic subunits. Of the aliphatic monomers,  $\omega$ -hydroxy- and  $\alpha,\omega$ -dicarboxylic acids (C<sub>16</sub>–C<sub>24</sub>) and C<sub>20</sub> to C<sub>30</sub> alcohols and acids predominate, while 20–60% of the suberin copolymer consists of aromatics [93]. Alkali-catalyzed methanolysis (i.e., heating in methanolic sodium methoxide) liberates hydroxy fatty acids (as their methyl ester) from both cutinized and suberized walls. All cell walls that contain cutin or suberin are also found to yield phenolic material on hydrolysis or methanolysis, and it has been widely accepted that the phenolic units are an integral part of cutin and suberin [166, 167]. No structure of the cutin or suberin polymers are available, but tentative models have been proposed by Kolattukudy et al. [166] (Fig. 2.43). Ferulic and *p*-coumaric



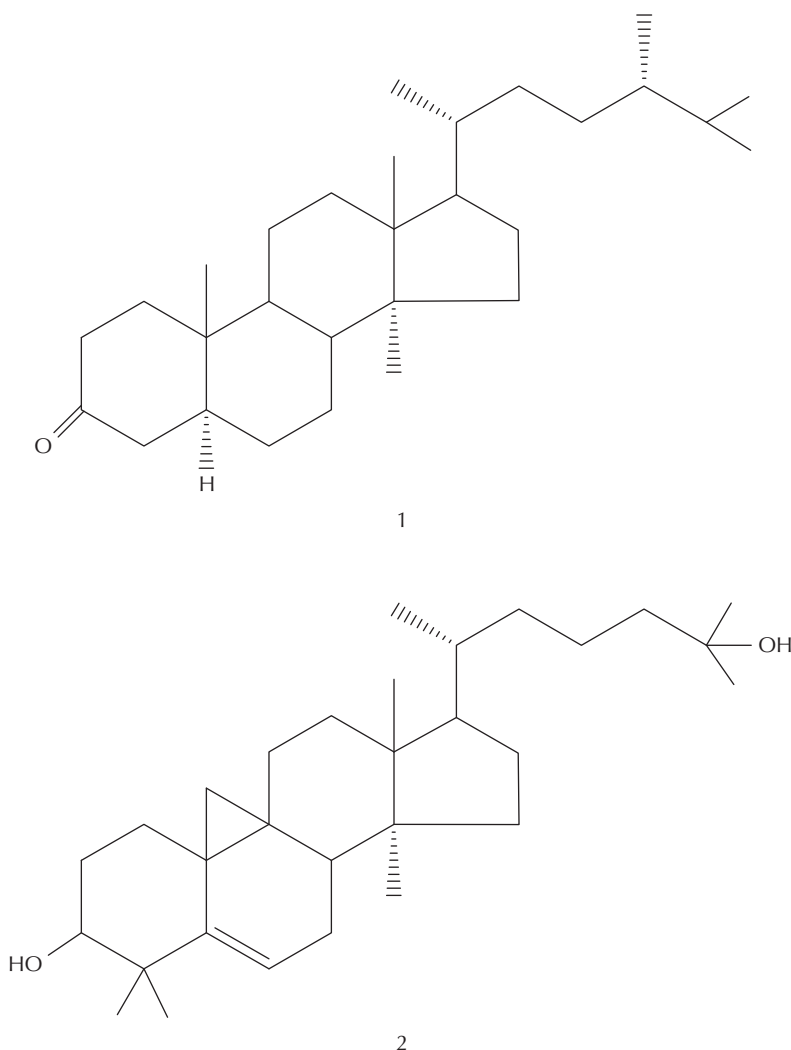
**FIGURE 2.43** Tentative structures of cutin and suberin [166].

acids have been found to be the constituents of cutins and suberins [166].

In addition to cutin and suberin, the third waterproofing group of compounds that have hitherto played no part in the harvest or postharvest economy is the plant waxes. Even though the content of wax in the straw crop comprised only 0.5–1.0% of the dry weight, this still constitutes 7.5–15 million tons of a valuable by-product of wax each year in the world. Most plants and all Gramineae are covered on both culm and leaf with semicrystalline wax particles 1–2  $\mu\text{m}$  across and 2–3  $\mu\text{m}$  high. These waxes are mainly long-chain alkane, ester and alcohol waxes [169]. Esters and fatty acids are common but usually minor components, whereas fatty alcohols more often comprise a major portion of the wax. The hydrocarbons of suberin associated waxes seem to have shorter average length and have larger proportions of even-chain lengths than cuticular alkanes [166]. The effect of this wax layer is to prevent water loss from the young plant and incidentally to help

shed natural or man-made liquids thrown at the leaf's surface [169].

Atldn et al. [170] has studied the surfaces of two major crop species, *Avena sativa* (oat) and *Sorghum bicolor* (sorghum), by a multidimensional approach, and identified the fractions containing the hydrocarbons, esters, aldehydes, and sterols and triterpenes. Neves and Gaspar [171, 172] reported that the extract composition of wheat (*Triticum aestivum*) straw was assessed as a complex mixture of organic acids and sterolic compounds. Further analyses as methyl derivatives and free hydroxyl groups identified about 20 compounds, most of them being phenolic acids. Naphthoic acid, azelaic acid, and 1, 2, 3, 5-tetrabromobenzene were also identified. From the neutral fraction of the acetone extract of mature wheat straw (*Triticum aestivum*), the following compounds have been identified: two new steroid ketones (24R)-14 $\alpha$ -methyl-5 $\alpha$ -ergostan-3-one and 14 $\alpha$ -methyl-5 $\alpha$ -cholestan-3-one; two new tetracyclic triterpenoids cycloart-5-ene-3 $\beta$ , 25-diol and cycloart-3 $\beta$ , 25-diol



**FIGURE 2.44** Structures of (24R)-14 $\alpha$ -methyl-5 $\alpha$ -ergostan-3-one; 1 (steroid ketone) and cycloart-5-ene-3 $\beta$ , 25-diol; 2 (tetracyclic triterpenoid) [173].



(Fig. 2.44); and already known compounds cholesterol, ergosterol, campesterol, stigmasterol, sitosterol, spinasterol, and sfigmastanol [173].

### 2.4.7 Ash

The minerals are known to vary widely depending on the agronomical factor and the amount of contaminated soil [118]. There are 19 minerals that are essential or useful for plant growth and development. The macronutrients, such as N, P, S, K, Mg, and Ca are integral to organic substances such as proteins and nucleic acids and maintain osmotic pressure. Their concentrations in plants vary from 0.1 to 1.5% of dry matter. The concentration of a particular mineral substance in a plant varies depending up on plant age or stage of development, plant species, and the concentration of other minerals [174] as well as the plant part. The micronutrients, such as Fe, Mn, Zn, Cu, B, Mo, Cl, and Ni, contribute mainly to enzyme production or activation and their concentrations in plants are low. The important minerals in cell wall are calcium, phosphorus and, in monocots, silica [175]. The latter can diminish the nutritive value [152]. Calcium can serve as a cross-linking agent in pectins, which is more important in dicots where the content of pectins is much higher [93]. The predominating elements of barley straw are silicon, potassium, calcium, and chlorine [176].

In general, ash content of fibrous raw material is between 1% and 20% [177], while the softwood and hardwoods contain almost negligible amount of minerals. However, all the agricultural fibers are rich in mineral matters. Of the total mineral content, 65–70% constitutes  $\text{SiO}_2$ . The role of silicon in plant cell wall is even more ambiguous. It has been reported that silicon has a positive effect on plant growth [178], contributes to the plant resistance against insects and fungi [179], and increases mechanical resistance [180]. Furthermore, silica has also been reported to be a constituent of certain glucosaminoglycans and polyuronides, where it is firmly bound to the polysaccharide organic matrix [181]. One could speculate that silicon could function somewhat in the same manner as lignin and bound with phenolic acids, as reticulating agent strengthening in grass cell walls. Until now, however, no clear evidence has been shown for the occurrence of cell wall-linked silicon derivatives [182, 183]. However, in the pulping process, the minerals of the raw material are considered to be impurities and should be removed during pulping or bleaching. It has been reported that silica content in the black liquors is in the order of 4–8% in wheat straw liquor, 16–30% in rice straw liquor, 2–8% in the case of reed straw, and 1–2% in the case of bagasse [184]. The chemical pulping of these raw materials presents two major problems: silica causes rather serious difficulties during recovery processes and slow drainage of straw pulp during

papermaking. Meanwhile, the presence of silica in the black liquor continues to cause problems all the way to the end of recovery sequence [177]. To solve this problem, as mentioned earlier, some desilication processes have been proposed either by removal of silica in the weak black liquor prior to evaporation or by desilication in the green liquor.

## REFERENCES

- [1] D. Fengel, G. Wegener, *Wood: Chemistry, Ultrastructure, Reactions*, Walter de Gruyter, Berlin, 1989.
- [2] E. Sjöström, *Wood Chemistry: Fundamentals and Applications*, second ed., Academic Press Inc., San Diego, 1993.
- [3] R.H. Newman, Homogeneity in cellulose crystallinity between samples of *Pinus radiata* wood, *Holzforschung* 58 (2004) 91–96.
- [4] U.P. Agarwal, Raman imaging to investigate ultrastructure and composition of plant cell walls: distribution of lignin and cellulose in black spruce wood (*Picea mariana*), *Planta* 224 (2006) 1141–1153.
- [5] F. Xu, X.C. Zhong, R.C. Sun, Q. Lu, Anatomy, ultrastructure and lignin distribution in cell wall of *Caragana Korshinskii*, *Ind. Crops. Prod.* 24 (2006) 186–193.
- [6] F. Xu, R.C. Sun, Q. Lu, G.L. Jones, Comparative study of anatomy and lignin distribution in normal and tension wood of *Salix gordejecii*, *Wood Sci. Technol.* 40 (2006) 358–370.
- [7] F. Xu, G.L. Jones, R.C. Sun, Fiber morphology and anatomical structure of sandlive willow (*Salix psammophila*), *Chem. Ind. Forest Prod.* 26 (2006) 91–94.
- [8] F. Xu, X.C. Zhong, R.C. Sun, Q. Lu, Anatomical features and ultrastructure of *Hedysarum scoparium*, *Trans. China Pulp Pap.* 21 (2006) 1–4.
- [9] M.S. Ilvessalo Pfäffli, *Fiber atlas, identification of papermaking fibers*, Springer Verlag, Berlin, 1995.
- [10] The Pulp and Paper Group at Norwegian University of Science and Technology, Non woods as paper raw material, [http://www.chemeng.ntnu.no/research/paper/Online\\_articles/Nonwoods/Nonwood.html](http://www.chemeng.ntnu.no/research/paper/Online_articles/Nonwoods/Nonwood.html), Norwegian University of Science and Technology, 05 Jul 2001.
- [11] T. Michaels, Plant Propagation: Lecture 7 Roots and stems, <http://blog.lib.umn.edu/michaels/fall09courseguide/Lecture%207%20%202008%20%20Roots%20and%20Stems.html>, University of Minnesota, 15 Jun 2009.
- [12] R.F. Evert, *Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body their Structure, Function, and Development*, third ed., John Wiley & Sons, New Jersey, 2006.
- [13] W.A. Côté, *Wood Ultrastructure*, University of Washington Press, New York, 1967.
- [14] D. Fengel, M. Stoll, Über die Veränderungen des Zellquerschnitts, der Dicke der Zellwand und der Wandschichten von Fichtenholz Tracheiden innerhalb eines Jahres, *Holzforschung* 27 (1973) 1–7.
- [15] H. Harada, Electron microscopy of ultrathin sections of beech wood (*Fagus crenata* Blume), *J. Japan Wood Res. Soc.* 8 (1962) 252–258.
- [16] J.D. Boyd, Helical fissures in compression wood cells: causative factors and mechanics of development, *Wood Sci. Technol.* 7 (1973) 92–111.
- [17] W. Liese, Tertiary wall and warty layer in wood cells, *J. Polym. Sci.* 2 (1963) 213–219.
- [18] J. Cronshaw, The formation of the wart structure in tracheids of *Pinus radiata*, *Protoplasma* 60 (1965) 233–242.

- [19] D.N.S. Hon, N. Shiraishi, Wood and cellulosic chemistry, second ed., revised and expanded, Marcel Dekker, New York, 2001.
- [20] J.R. Barnett, V.A. Bonham, Cellulose microfibril angle in the cell wall of wood fibers, *Biol. Rev.* 79 (2004) 461–472.
- [21] J.C.F. Walker, Primary Wood Processing, Principles and Practice, second ed., University of Canterbury Christchurch, New Zealand, 2006.
- [22] A.B. Wardrop, The structure and formation of the cell wall in xylem, in: M.H. Zimmermann (Ed.), *The Formation of Wood in Forest Trees*, Academic Press Inc., New York, 1964, pp. 87–134.
- [23] H. Harada, W.A. Côté, Structure of wood, in: T. Higuchi (Ed.), *Biosynthesis and Biodegradation of Wood Components*, Academic Press, Orlando, 1985, pp. 1–42.
- [24] J. Brandstrom, S.L. Bardage, G. Daniel, T. Nilsson, The structural organization of the S1 cell wall layer of Norway spruce tracheids, *IAWA J.* 24 (2003) 27–40.
- [25] H. Abe, R. Funada, Review The orientation of cellulose microfibrils in the cell walls of tracheids in conifers, *IAWA J.* 26 (2005) 161–174.
- [26] H. Abe, R. Funada, J. Ohtani, K. Fukazawa, Changes in the arrangement of microtubules and microfibrils in differentiating conifer tracheids during the expansion of cells, *Ann. Bot.* 75 (1995) 305–310.
- [27] M.C. Probine, The plant cell wall, *Tuatara*, 11(1963) 115–129. [http://www.nzetc.org/tm/scholarly/Bio11Tuat02\\_fig\\_Bio11Tuat02\\_129a.html](http://www.nzetc.org/tm/scholarly/Bio11Tuat02_fig_Bio11Tuat02_129a.html), Victoria University of Wellington, 2008.
- [28] S. Stuart, R. Evans, X ray diffraction estimation of the microfibril angle variation in eucalypt wood, *Appita J.* 48 (1994) 197–200.
- [29] C.W. McMillin, Fibril angle of loblolly pine wood as related to specific gravity, growth rate, and distance from pith, *Wood Sci. Technol.* 7 (1973) 251–255.
- [30] U.P. Agarwal, S.A. Ralph, Revealing organization of cellulose in wood cell walls by Raman imaging, in: *Proceedings of the 14th international symposium on wood fiber and pulping chemistry in Durban, South Africa, 2007*, pp. 1–6.
- [31] A. Frey Wyssling, *Die Pflanzliche Zellwand*, Springer, Berlin Heidelberg New York, 1959.
- [32] B.L. Browning, *Methods of Wood Chemistry*, vol. II, Interscience Publishers, New York, USA, 1967.
- [33] R.B. Hanna, W.A. Côté, The sub elementary fibril of plant cell wall cellulose, *Cytobiology* 10 (1974) 102–116.
- [34] A.J. Kerr, D.A.I. Goring, The ultrastructural arrangement of the wood cell wall, *Cell. Chem. Technol.* 9 (1975) 563–573.
- [35] Ceres, Carbohydrates: The More Carbs the better, <http://www.ceres.net/AboutUs/AboutUs Biofuels Carbo.html> Ceres, Inc., 2007.
- [36] H.M. Zhai, Z.Z. Lee, Ultrastructure and topochemistry of delignification in alkaline pulping of wheat straw, *J. Wood Chem. Technol.* 9 (1989) 387–406.
- [37] Z.Z. Lee, Pulping principles of wheat straw, *China Pulp Pap. J.* 1 (1986) 24–28.
- [38] J.H. Wang, Y.H. Li, W.Y. Meng, Ultrastructure lignin and silicon distribution in fiber walls of reed, in: *Proceeding of the 1980 annual conference of China Academy of Papermaking*, Beijing, China, 1980, pp. 1–15.
- [39] N. Parameswaran, W. Liese, On the fine structure of bamboo fibers, *Wood Sci. Technol.* 10 (1976) 231–246.
- [40] H.P.S. Abdul Khalil, M. Siti Alwani, A.K. Mohd Omar, Chemical composition, anatomy, lignin distribution, and cell wall structure of Malaysian plant waste fibers, *Bioresources* 1 (2006) 220–232.
- [41] R.E. Booker, J. Sell, The nanostructure of the cell wall of softwoods and its function in a living tree, *Holz Roh. Werkst.* 56 (1998) 1–8.
- [42] A. Singh, G. Daniel, T. Nilsson, High variability in the thickness of the S3 layer in *Pinus radiata* tracheids, *Holzforschung* 56 (2002) 111–116.
- [43] H. Meier, The distribution of polysaccharides in wood fibers, *J. Polym. Sci.* 51 (1961) 11–18.
- [44] R.H. Atalla, U.P. Agarwal, Raman microprobe evidence for lignin orientation in the cell walls of native woody tissue, *Science* 227 (1985) 636–638.
- [45] U.P. Agarwal, R.H. Atalla, In situ Raman microprobe studies of plant cell walls: macromolecular organization and compositional variability in the secondary wall of *Picea mariana* (Mill.) B.S.P., *Planta* 169 (1986) 325–332.
- [46] J.S. Bond, R.H. Atalla, A Raman microprobe investigation of the molecular architecture of loblolly pine tracheids, in: *Proceedings of 10th international symposium on wood pulp chemistry*, vol. 1, Tappi Press, Atlanta, 1999, pp. 96–101.
- [47] V.C. Tirumalai, U.P. Agarwal, J.R.O. Obst, Heterogeneity of lignin concentration in cell corner middle lamella of white birch and black spruce, *Wood Sci. Technol.* 30 (1996) 99–104.
- [48] H. Meier, Localization of polysaccharides in wood cell walls, in: T. Higuchi (Ed.), *Biosynthesis and Biodegradation of Wood Components*, Academic Press Inc., Orlando, 1985, pp. 43–50.
- [49] J.D. Pickett Heaps, Preliminary attempts at ultrastructural polysaccharide localization in root tip cells, *J. Histochem. Cytochem.* 15 (1967) 442–455.
- [50] J.D. Pickett Heaps, Further ultrastructural observation on polysaccharide localization in plant cells, *J. Cell. Sci.* 3 (1968) 55–64.
- [51] A. Freundlich, A.W. Robards, Cytochemistry of differentiating plant vascular cell walls with special reference to cellulose, *Cytobiologie* 8 (1974) 355–370.
- [52] P. Mbersheim, K. Mühlethaler, A. Frey Wyssling, Stained pectins as seen in the electron microscope, *J. Biophys. Biochem. Cytol.* 8 (1960) 501–506.
- [53] P.M. Colombo, N. Rascio, Ruthenium red staining for electron microscopy of plant material, *J. Ultrastruct. Res.* 60 (1977) 135–139.
- [54] M. Rougier, B. Vian, D. Gallant, J.C. Roland, Aspects cytochimiques de l'étude ultrastructurale des polysaccharides végétaux, *Ann. Biol.* 12 (1973) 43–75.
- [55] M. Sinner, N. Parameswaran, H. Dietrichs, Degradation of delignified spruce wood by purified mannanase, xylanase and cellulases, in: R.D. Brown Jr., L. Jurasek (Eds.), *Hydrolysis of cellulose mechanisms of enzymatic and acid catalysis*, Am. Chem. Soc., Washington DC, 1979, pp. 303–329.
- [56] G. Cox, B. Juniper, Electron microscopy of cellulose in entire tissue, *J. Microsc.* 97 (1973) 343–355.
- [57] R.H. Mullis, N.S. Thompson, R.A. Parham, The localization of pentosans within the cell wall of aspen (*Populus tremuloides* Michx.) by high resolution autoradiography, *Planta* 132 (1976) 241–248.
- [58] P. Hoffmann, N. Parameswaran, On the ultrastructural localization of hemicelluloses within delignified tracheids of spruce, *Holz forschung* 30 (1976) 62–70.
- [59] H. Meier, Chemical and morphological aspects of the fine structure of wood, *Pure Appl. Chem.* 5 (1962) 37–52.
- [60] K. Takabe, Deposition of cell wall components in conifer tracheids, PhD thesis, Dept. Wood Science Technology, Kyoto Univ., Kyoto, Japan, 1984.
- [61] N. Parameswaran, W. Liese, Ultrastructural localization of wall components in wood cells, *Holz Roh. Werkst.* 40 (1982) 145–155.

- [62] C. Mäule, Das Verhalten verholzter Membranen gegen Kaliumpermanganat, eine Holzreaktion neuer Art, Beiträge zur Wiss., Botanik 4 (1900) 166–185.
- [63] T.E. Timell, Studies on opposite wood in conifers part III: distribution of lignin, Wood Sci. Technol. 12 (1973) 1–15.
- [64] P.W. Lange, The distribution of lignin in the cell wall of normal and reaction wood from spruce and a few hardwoods, Sven. Papperstidn. 57 (1954) 525–532.
- [65] B.J. Fergus, D.A.I. Goring, The topochemistry of delignification in kraft and neutral sulfite pulping of birch wood, Pulp Pap. Mag. Can. 70 (1969) 314–322.
- [66] J.R. Wood, D.A.I. Goring, The distribution of lignin in stem wood and branch wood of Douglas fir, Pulp Pap. Mag. Can. 72 (1971) 61–68.
- [67] P.W. Lange, A. Kjaer, Quantitative chemical analysis of the different parts of the cell wall in wood and cellulose fibers by interference microscopy, Norsk. Skogind. 11 (1957) 425–432.
- [68] J.B. Boutelje, B.M. Hollmark, Studies with the interference microscopy on enzymatic hydrolysis of fiber wall, Holzforschung 26 (1972) 76–81.
- [69] L.A. Donaldson, K.G. Ryan, A comparison of relative lignin concentration as determined by interference microscopy and bromination/EDXA, Wood Sci. Technol. 21 (1987) 303–309.
- [70] S. Saka, D.A.I. Goring, Distribution of lignin in white birch wood as determined by bromination with TEM EDXA, Holzforschung 42 (1988) 149–153.
- [71] I. Eriksson, O. Lindbrandt, U. Westermarck, Lignin distribution in birch (*Betula verrucosa*) determined by mercerization with SEM and TEM EDXA, Wood Sci. Technol. 22 (1988) 251–257.
- [72] U. Westermarck, O. Lindbrandt, I. Eriksson, Lignin distribution in spruce (*Picea abies*) determined by mercerization with SEM EDXA technique, Wood Sci. Technol. 22 (1988) 243–250.
- [73] L.A. Donaldson, Determination of lignin distribution in agricultural fibers, Wood Processing Division, New Zealand Forest Research Institute 4418 (1996) 1–25.
- [74] M. Fujita, H. Harada, Autoradiographic investigation of cell wall development. II. Tritiated phenylalanine and ferulic acid assimilation in relation to lignification, Mokuzai Gakkaishi 25 (1979) 89–94.
- [75] L.A. Donaldson, Lignification and lignin topochemistry: an ultrastructural view, Phytochemistry 57 (2001) 859–873.
- [76] S. Saka, R.J. Thomas, A study of lignification in loblolly pine tracheids by the SEM EDXA technique, Wood Sci. Technol. 16 (1982) 167–179.
- [77] S. Saka, R.J. Thomas, J.S. Gratzl, D. Abson, Topochemistry of delignification in Douglas fir wood with soda, soda anthraquinone and kraft pulping as determined by SEM EDXA, Wood Sci. Technol. 16 (1982) 139–153.
- [78] D. Nilsson, Studies on the S2 layer of *Pinus sylvestris* L., Swedish University of Agricultural Sciences, Uppsala, Sweden, Report no. 154, 1984, pp. 1–34.
- [79] A. Maurer, D. Fengel, Electron microscopic representation of structural details in softwood cell walls by very thin ultramicrotome sections, Holz Roh. Werkst. 49 (1991) 53–56.
- [80] L.A. Donaldson, Cell wall fracture properties in relation to lignin distribution and cell dimensions among three genetic groups of radiata pine, Wood Sci. Technol. 29 (1995) 51–63.
- [81] J.A.N. Scott, D.A.I. Goring, Lignin concentration on the S3 layer of softwoods, Cell. Chem. Technol. 4 (1970) 83–93.
- [82] F. Xu, R.C. Sun, X.C. Zhong, Ultrastructure and lignin distribution in cell wall of *Caragana Korshinskii*, Abstracts of papers of the American Chemistry Society, 231st ACS National Meeting, Atlanta, GA, United States, 63 CELL, 2006.
- [83] F. Xu, X.C. Zhong, R.C. Sun, G.L. Jones, Lignin distribution and ultrastructure of *Salix psammophila*, Trans. China Pulp Pap. 20 (2005) 6–9.
- [84] B.J. Fergus, D.A.I. Goring, The distribution of lignin in birch wood as determined by ultraviolet microscopy, Holzforschung 24 (1970) 118–124.
- [85] L.A. Donaldson, J. Hague, R. Snell, Lignin distribution in coppice poplar, linseed and wheat straw, Holzforschung 55 (2001) 379–385.
- [86] S.G. Wi, A.P. Singh, K.H. Lee, Y.S. Kim, The pattern of distribution of pectin, peroxidase and lignin in the middle lamella of secondary xylem fibers in alfalfa (*Medicago sativa*), Ann. Bot. 95 (2005) 863–868.
- [87] B. Lybeer, G. Koch, Lignin distribution in the tropical bamboo species *Gigantochloa levis*, IAWA J. 26 (2005) 443–456.
- [88] I.M. Morrison, The degradation and utilization of straw in the rumen, in: E. Grossbard (Ed.), Straw Decay and its Effect on Disposal and Utilization, Wiley, Chichester, 1979, pp. 237–245.
- [89] R.C. Sun, T. Tomkinson, Essential guides for isolation/purification of polysaccharides, in: I.D. Wilson, T.R. Adlard, C.F. Poole, M. Cook (Eds.), Encyclopedia of Separation Science, Academic Press, London, 2000, pp. 4568–4574.
- [90] S.H.T. Harper, J.M. Lynch, The chemical components and decomposition of wheat straw leaves, internodes and nodes, J. Sci. Food Agric. 32 (1981) 1057–1062.
- [91] O. Theander, Review of straw carbohydrate research, in: R.D. Hill, L. Munck (Eds.), New Approaches to Research on Cereal Carbohydrates, Elsevier Science Publishers B. V., Amsterdam, 1985, pp. 217–230.
- [92] P. Albersheim, A.G. Darvill, K.R. Davis, J.M. Lau, M. McNeil, J.K. Sharp, W.S. York, Why study the structures of biological molecules? in: W.M. Dugger, S. Bartnicki Garcia (Eds.), Structure, Function and Biosynthesis of Plant Cell Walls, American Society of Plant Physiologists, Rockville, 1984, pp. 19–51.
- [93] E. Nordkvist, Composition and degradation of cell walls in red clover, lucerne and cereal straw, PhD thesis, The Swedish University of Agricultural Sciences, Uppsala, Sweden, 1987.
- [94] E. Percival, R.H. McDowell, Algal walls: composition and biosynthesis, in: W. Tanner, F.A. Loewus (Eds.), Encyclopedia of Plant Physiology, vol. 13B, Plant Carbohydrates II, Extracellular Carbohydrates, Springer, Berlin, 1981, pp. 277–316.
- [95] P.J. Evans, Chemical and physical aspects of the interaction of sodium hydroxide with the cell wall components of straw, in: E. Grossbard (Ed.), Straw Decay and its Effect on Disposal and Utilization, University of Research Ltd, Sharnbrook, Bedford, 1979, pp. 187–197.
- [96] M. Marx Figini, Investigations on biosynthesis of cellulose: DP<sub>w</sub> and yield of the alga *Volonia* in the presence of colchicines, Biochim. Biophys. Acta 237 (1971) 75–77.
- [97] G. Franz, Biosynthesis of cellulose and semi celluloses, Papier 32 (1978) 521–525.
- [98] R.G. Zbankov, Hydrogen bonds and structure of carbohydrates, J. Mol. Struct. 270 (1992) 523–539.
- [99] Y. Nishiyama, P. Langan, H. Chanzy, Crystal structure and hydrogen bonding system in cellulose I<sub>β</sub> from synchrotron X ray and neutron fiber diffraction, J. Am. Chem. Soc. 124 (2002) 9074–9082.
- [100] J.F. Matthews, C.E. Skopeck, P.E. Mason, P. Zuccato, R.W. Torget, J. Sugiyama, M.E. Himmel, J.W. Brady, Computer simulation

- studies of microcrystalline cellulose I beta, *Carbohydr. Res.* 341 (2006) 138–152.
- [101] G.O. Aspinall, Structural chemistry of the hemicelluloses, *Adv. Carbohydr. Chem. Bi.* 14 (1959) 429–468.
- [102] Z.S. Cai, L. Paszner, Salt catalyzed wood bonding with hemicellulose, *Holzforschung* 42 (1988) 11–12.
- [103] R.L. Whistler, Xylan, *Adv. Carbohydr. Chem. Bi.* 5 (1950) 269–290.
- [104] G.O. Aspinall, R.S. Mahomed, The constitution of a wheat straw xylan, *J. Am. Chem. Soc.* 76 (1954) 1731–1734.
- [105] I. Ehrenthal, R. Montgomery, F. Smith, The carbohydrates of Gramineae. II. The constitution of the hemicelluloses of wheat straw and corn cobs, *J. Am. Chem. Soc.* 76 (1954) 5509–5514.
- [106] C.P.J. Glauermanas, T.E. Tixell, The polysaccharides of White Birch (*Betula papyrifera*). IV. The constitution of the hemicellulose, *J. Am. Chem. Soc.* 80 (1958) 1209–1213.
- [107] T.E. Timell, Wood hemicelluloses I., *Adv. Carbohydr. Chem. Bi.* 19 (1964) 247–302.
- [108] T.E. Timell, Wood hemicelluloses II., *Adv. Carbohydr. Chem. Bi.* 20 (1965) 409–483.
- [109] I.M. Morrison, Changes in the hemicellulosic polysaccharides of rye grass with increasing maturity, *Carbohydr. Res.* 36 (1974) 45–51.
- [110] G.O. Aspinall, Chemistry of cell wall polysaccharides, in: J. Presis (Ed.), *The Biochemistry of Plants*, Academic Press, New York, 1980, pp. 473–500.
- [111] A.J. Buchala, C.G. Fraser, K.C.B. Wilkie, An acidic galactoarabinoxylan from the stem of *Avena sativa*, *Phytochemistry* 11 (1972) 2803–2814.
- [112] J.S.D. Bacon, A.H. Gordon, E.J. Morris, Acetyl groups in cell wall preparations from higher plants, *Biochem. J.* 149 (1975) 485–487.
- [113] R.D. Hartley, Carbohydrate esters of ferulic acid as components of cell walls of *Lolium multiflorum*, *Phytochemistry* 12 (1973) 661–665.
- [114] I. Mueller Harvey, R.D. Hartley, Linkage of *p* coumaroyl and feruloyl groups to cell wall polysaccharides of barley straw, *Carbohydr. Res.* 148 (1986) 71–85.
- [115] A.G. Darvill, M. McNeil, P. Albersheim, D.P. Delmer, The primary cell walls of flowering plants, in: P.K. Stumpf, E.E. Conn (Eds.), *The Biochemistry of Plants: A Comprehensive Treatise*, Academic Press, New York, 1980, pp. 91–162.
- [116] G.O. Aspinall, Constitution of plant cell wall polysaccharides, in: W. Tanner, F.A. Loewus (Eds.), *Plant Carbohydrates*, 2. Extra cellular Carbohydrates, Springer Verlag, Heidelberg, 1981, pp. 3–8.
- [117] G.C. Ruben, G.H. Bokelman, Triple stranded, left hand twisted cellulose microfibril, *Carbohydr. Res.* 160 (1987) 434–443.
- [118] I. Ternrud, Degradation of untreated and alkali treated straw polysaccharides in ruminants, PhD thesis, The Swedish University of Agricultural Sciences, Uppsala, Sweden, 1987.
- [119] A.J. Buchala, K.C.B. Wilkie, Non endospermic hemicellulose/ $\beta$  Glucans from cereal, *Naturwissenschaften* 57 (1970) 496–496.
- [120] I.S.D. Reid, K.C.B. Wilkie, Total hemicelluloses from oat plants at different stages of growth, *Phytochemistry* 8 (1969) 2059–2065.
- [121] Y. Kato, D.J. Nevins, Fine structure of (1 $\rightarrow$ 3), (1 $\rightarrow$ 4)  $\beta$  D glucan from *Zea* shoot cell walls, *Carbohydr. Res.* 147 (1986) 69–85.
- [122] R.C. Sun, J.M. Lawther, W.B. Banks, Fractional and structural characterization of wheat straw hemicelluloses, *Carbohydr. Polym.* 29 (1996) 325–331.
- [123] K.V. Sarkanen, C.H. Ludwig, Definition and nomenclature, in: K.V. Sarkanen, C.H. Ludwig (Eds.), *Lignins, Occurrence, Formation, Structure and Reactions*, Wiley Interscience, New York, 1971, pp. 1–18.
- [124] J.J. MacKay, D.M. O'Malley, T. Presnell, F.L. Booker, M.M. Campbell, R.W. Whetten, R.R. Sederoff, Inheritance, gene expression, and lignin characterization in a mutant pine deficient in cinnamyl alcohol dehydrogenase, *Proc. Natl. Acad. Sci. USA* 94 (1997) 8255–8260.
- [125] J. Ralph, J.J. MacKay, R.D. Hatfield, D.M. O'Malley, R.W. Whetten, R.R. Sederoff, Abnormal lignin in a loblolly pine mutant, *Science* 277 (1997) 235–239.
- [126] J. Ralph, R.D. Hatfield, J. Piquemal, N. Yahiaoui, M. Pean, C. Lapierre, A. M. Boudet, NMR characterization of altered lignins extracted from tobacco plants down regulated for lignification enzymes cinnamylalcohol dehydrogenase and cinnamoyl CoA reductase, *Proc. Natl. Acad. Sci.* 95 (1998) 12803–12808.
- [127] C. Halpin, M.E. Knight, G.A. Foxon, M.M. Campbell, A.M. Boudet, J.J. Boon, B. Chabbert, M. T. Tollier, W. Schuch, Manipulation of lignin quality by downregulation of cinnamyl alcohol dehydrogenase, *Plant J.* 6 (1994) 339–350.
- [128] M. Baucher, B. Chabbert, G. Pilate, J. van Doorselaere, M. T. Tollier, M. Petit Conil, D. Cornu, B. Monties, van Montagu, D. Inze, Red xylem and higher lignin extractability by down regulating cinnamyl alcohol dehydrogenase in poplar (*Populus tremula*  $\times$  *Populus alba*), *Plant Physiol.* 112 (1996) 1479–1490.
- [129] R.C. Sun, P. Rowlands, J.M. Lawther, Rapid isolation and physico chemical characterization of wheat straw lignins, *Recent Res. Dev. Agric. Food Chem.* 4 (2000) 1–26.
- [130] D. Fengel, G. Wegerner, *Wood: Chemistry, Ultrastructure, Reactions*, Walter de Gruyter, Berlin, 1984.
- [131] K.C.B. Wilkie, The hemicelluloses of grasses and cereals, *Adv. Carbohydr. Chem. Biochem.* 36 (1979) 215–264.
- [132] T. Higuchi, Lignin biochemistry: biosynthesis and biodegradation, *Wood Sci. Technol.* 24 (1990) 23–63.
- [133] B.J. Fergus, A.R. Procter, J.A.N. Scott, D.A.I. Goring, The distribution of lignin in sprucewood as determined by ultraviolet microscopy, *Wood Sci. Technol.* 3 (1969) 117–138.
- [134] G. Wallace, S.C. Fry, Phenolic components of the plant cell wall, *Int. Rev. Cytol.* 151 (1994) 229–267.
- [135] A. Björkman, Studies on finely divided wood. 3. Extraction of lignin carbohydrate complexes with neutral solvents, *Sven. Papperstidn.* 60 (1957) 243–251.
- [136] C.W. Ford, Comparative structural studies of lignin carbohydrate complexes from *Digitaria decumbens* (pangola grass) before and after chlorite delignification, *Carbohydr. Res.* 147 (1986) 101–107.
- [137] S.I. Falkehag, Synthesis of phenolic polymer, *Appl. Polym. Symp.* 28 (1975) 247–257.
- [138] M.L. Fidalgo, M.C. Terron, A.T. Martinez, A.E. Gonzalez, F.J. Gonzalez vila, G.C. Galletti, Comparative study of fractions from alkaline extraction of wheat straw through chemical degradation, analytical pyrolysis, and spectroscopic techniques, *J. Agric. Food Chem.* 41 (1993) 1621–1626.
- [139] A. Scalbert, B. Monties, J.Y. Lallemand, E. Guittet, C. Rolando, Ether linkage between phenolic acids and lignin fractions from wheat straw, *Phytochemistry* 24 (1985) 1359–1362.
- [140] R.C. Sun, J.M. Lawther, W.B. Banks, A tentative chemical structure of wheat straw lignin, *Ind. Crops. Prod.* 6 (1997) 1–8.
- [141] R.C. Sun, J.M. Lawther, W.B. Banks, Fractional isolation and physico chemical characterization of alkali soluble lignins from wheat straw, *Holzforschung* 51 (1997) 244–250.
- [142] E. Adler, J.M. Pepper, E. Eriksoo, Action of mineral acid on lignin and model substances of guaiacylglycerol beta aryl ether type, *Ind. Eng. Chem.* 49 (1957) 1391–1392.



- [143] B.F. Hrutfiord, Reduction and hydrogenolysis, in: K.V. Sarkanen, C.H. Ludwig (Eds.), *Lignins, Occurrence, Formation, Structure and Reactions*, Wiley Interscience, New York, 1971, pp. 487–509.
- [144] T.P. Schultz, T.H. Fisher, S.M. Dershem, Role of the *p* hydroxyl group in the nitrobenzene oxidation of hydroxybenzyl alcohols, *J. Org. Chem.* 52 (1987) 279–281.
- [145] J.M. Pepper, B.W. Casselman, J.C. Karapally, Lignin oxidation. Preferential use of cupric oxide, *Can. J. Chem.* 45 (1967) 3009–3012.
- [146] K. Freudenberg, The constitution and biosynthesis of lignin, in: K. Freudenberg, A.C. Neish (Eds.), *Constitution and Biosynthesis of Lignin*, Springer, Berlin, Heidelberg, New York, 1968, pp. 78–82.
- [147] C. Doree, M. Cunningham, The action of ozone on cellulose. III. Action on beech wood (lignocellulose), *J. Am. Chem. Soc.* 103 (1913) 677–686.
- [148] C. Lapiere, B. Monties, C. Rolando, Thioacidolysis of diazo methane methylated pine compression wood and wheat straw in situ lignins, *Holzforschung* 42 (1988) 409–411.
- [149] F.C. Lu, J. Ralph, DFRC method for lignin analysis. I. New method for  $\beta$  aryl ether cleavage: lignin model studies, *J. Agric. Food Chem.* 45 (1997) 4655–4660.
- [150] I.M. Morrison, A semi micro method for the determination of lignin and its use in predicting the digestibility of forages, *J. Sci. Food Agric.* 23 (1972) 455–463.
- [151] O. Theander, P. Åman, Studies on dietary fibers, *Swed. J. Agric. Res.* 9 (1979) 97–106.
- [152] P. Van Soest, R.H. Wine, Determination of lignin and cellulose in acid detergent fiber with permanganate, *J. Assoc. Off. Anal. Chem.* 51 (1968) 780–785.
- [153] M.W. Spellman, M. McNeil, A.G. Darvill, P. Albersheim, Isolation and characterization of 3 C carboxy 5 deoxy L xylose, a naturally occurring, branched chain, acidic monosaccharide, *Carbohydr. Res.* 122 (1983) 115–129.
- [154] M.C. Jarvis, Structure and properties of pectin gels in plant cell walls, *Plant Cell Environ.* 7 (1984) 153–164.
- [155] S.C. Fry, Feruloyated pectins from the primary cell wall: their structure and possible function, *Planta* 157 (1983) 111–123.
- [156] S.C. Fry, Isodityrosine, a new cross linking amino acid from plant cell wall glycoprotein, *Biochem. J.* 204 (1982) 449–455.
- [157] J.P. Knox, L. Jones, H.J.M. Thompson, W.G.T. Willats, E.A. Yates, Biological perspectives for plant carbohydrate chemistry, in: W. Mackie (Ed.), *The Royal Society of Chemistry Spring Meeting of the Carbohydrate Group*, University of Leeds, Leeds, 1995, pp. 19–21.
- [158] D.T.A. Lampion, Structure, biosynthesis and significance of cell wall glycoproteins, *Recent Adv. Phytochem.* 11 (1977) 79–115.
- [159] F. Meuser, P. Suckow, Non starch polysaccharides, in: J.M.V. Blanshard, P.J. Frazier, T. Galliard (Eds.), *Chemistry and Physics of Baking*, The Royal Society of Chemistry, London, 1986, pp. 42–61.
- [160] Y. Akiyama, K. Kato, Structure of hydroxyproline arabinoside from tobacco cells, *Agric. Biol. Chem.* 41 (1977) 79–81.
- [161] D.T.A. Lampion, D.H. Miller, Hydroxyproline arabinosides in the plant kingdom, *Plant Physiol.* 48 (1971) 454–456.
- [162] D.T.A. Lampion, L. Katona, S. Roerig, Galactosylserine in extensin, *Biochem. J.* 133 (1973) 125–131.
- [163] T. Ohmori, K. Tamura, K. Fukui, G. Kawanishi, M. Mitsuyama, K. Nomoto, T. Miyazaki, Isolation of galactosaminoglycan moiety (CO N) from protein bound polysaccharide of *Cordyceps ophioglossoides* and its effects against murine tumors, *Chem. Pharm. Bull.* 37 (1989) 1019–1022.
- [164] J.B. Cooper, J.E. Varner, Insolubilization of hydroxyproline rich cell wall glycoprotein in aerated carrot root slices, *Biochem. Biophys. Res. Commun.* 112 (1983) 161–167.
- [165] R. Toman, A. Chimidcogzol, L. Arabino (4 O methyl D glucurono) D xylan from the straw of the wheat Orchon (*Triticum bulgary Z.*), *Chem. Pap.* 42 (1988) 649–657.
- [166] P.E. Kolattukudy, K.E. Espelie, C.L. Soliday, Hydrophobic layers attached to cell walls: cutin, suberin, and associated waxes, in: W. Tanner, F.A. Loewus (Eds.), *Plant Carbohydrates*, Elsevier, Amsterdam, 1981, pp. 4–24.
- [167] A.J. Brown, P.E. Kolattukudy, Mammalian utilization of cutin, the cuticular polyester of plants, *J. Agric. Food Chem.* 26 (1978) 1263–1266.
- [168] P.E. Kolattukudy, Karen Kronman, A.J. Poulose, Determination of structure and composition of suberin from the roots of carrot, parsnip, tubataga, turnip, red beet and sweet potato by combined gas liquid chromatography and mass spectrometry, *Plant Physiol.* 55 (1975) 567–573.
- [169] B.E. Juniper, The structure and chemistry of straw, *Agric. Prog.* 54 (1979) 18–27.
- [170] D.S.J. Atldn, E.A. Bernays, R.J. Hamilton, *The Analysis of Plant Waxes*, Liverpool Polytechnic, England, 1990.
- [171] H.J.C.D. Neves, E.M.M. Gaspar, Identification of active compounds in wheat straw extracts with allelopathic activity by HRGC MS and HRGC FTIR, *J. High Resolut. Chromatogr.* 13 (1990) 550–554.
- [172] H.J.C.D. Neves, E.M.M. Gaspar, HRGC MS and HPLC MS identification of new ketosterols in an extract of wheat straw, *J. High Resolut. Chromatogr.* 18 (1995) 299–303.
- [173] E.M.M. Gaspar, H.J.C.D. Neves, Steroidal constituents from mature wheat straw, *Phytochemistry* 34 (1993) 523–527.
- [174] M. Gill, D.E. Beever, D.F. Osbourn, The feeding value of grass and grass products, in: W. Holmes (Ed.), *Grass, its Production and Utilization*, Blackwell Scientific Publications, Oxford, 1989, pp. 89–129.
- [175] W.R. McManus, R.G. Anthony, L.L. Grout, A.S. Malin, V.N.E. Robinson, Biocrystallization of plant material on forage plant cell walls, *Aust. J. Agric. Res.* 30 (1979) 635–649.
- [176] O. Theander, P. Åman, Anatomical and chemical characteristics, in: F. Sundstøl, E. Owen (Eds.), *Straw and other Fibrous By Products as Feed*, Elsevier, Amsterdam, 1984, pp. 45–78.
- [177] H. Eroglu, I. Deniz, Predesilication of wheat straw with NaOH, *Das Papier* 11 (1993) 645–650.
- [178] J. Ma, E. Takahashi, Effect of silicon on the growth and phosphorous uptake of flue, *Plant Soil* 126 (1990) 115–119.
- [179] A.G. Sangster, M.J. Hondson, Silica in higher plants, in: D. Evered, M. O'Connor (Eds.), *Silicon Biochemistry*, Wiley, Chichester, 1986, pp. 90–111.
- [180] R.K. Iler, *The Chemistry of Silica*, Wiley, New York, 1979.
- [181] K. Schwarz, A bound form of silicon in glucosaminoglucans and polyuronides, *Proc. Natl. Acad. Sci. USA* 70 (1973) 1608–1612.
- [182] E. Billa, B. Monties, Occurrence of silicon associated with lignin polysaccharide complexes isolated from gramineae (wheat straw) cell walls, *Food Hydrocolloids* 5 (1991) 189–195.
- [183] R.J.P. Williams, Introduction to silicon chemistry and biochemistry, in: D. Evered, M. O'Connor (Eds.), *Silicon Biochemistry*, Wiley, Chichester, 1986, pp. 24–39.
- [184] J. Salaber, F. Maza, Bagasse black liquor recovery, *Filtr. Sep.* 9 (1972) 55–77.

# Extractives

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The extractives are a heterogeneous group of substances, which can be extracted from wood or straw using solvents like acetone or dichloromethane. The main extractives are resin acids, triglycerides, steryl esters, fatty acids, sterols, neutral compounds, such as fatty alcohols, sterols, and a variety of phenolic compounds [1]. Importantly, the chemical composition is dependent on the factors that influence the wood or nonwood species, such as straw, growing age, and environmental conditions [2]. Although extractives are a minor component, often constituting less than 2% of the total dry matter, they make a major contribution to the characteristics of wood or nonwood species. In general, these extractives are low-molecular-weight compounds, and the different classes of extractives have different chemical behaviors. Considerable amounts of the extractives can be dispersed or dissolved during mechanical fibrillation and mechanical pulp bleaching operations [3]. However, these dispersed extractives are difficult to remove and can cause pith problems during pulping and papermaking. In neutral and acidic processes, it is difficult to remove the lipophilic extractives. During alkaline processes, such as kraft pulping, fats and certain waxes are completely saponified, and the fatty acids and resin acids dissolve as soluble soaps. However, sterols and some waxes do not form soluble soap under alkaline conditions and therefore have a tendency to deposit and cause pitch problems [4]. Consequently, in papermaking, the accumulation of these extractives causes significant technical and economic problems for pulp and paper manufacturers [5]. On the other hand, pitch problems are likely to become more severe with the introduction of more environmentally friendly bleaching processes that had substituted chlorine gas with other reagents such as chlorine dioxide, hydrogen peroxide, or ozone. Likewise, the increasing reuse of white water and the trend toward complete closure of water circuits to meet environmental protection requirements are leading to an increase in

pitch concentrations, which would result in a higher deposition [4].

Straw is one of the main agricultural residues and is produced in large quantities worldwide every year. Among the residues, wheat, barley, rye, and rice straws are the most economically important raw materials and are used extensively for pulp and paper production in developing countries, particularly Algeria, Argentina, China, and India. In these countries, corrugating medium, board, and packaging paper are produced from high-yield unbleached straw pulps, whereas bleached straw pulps are used as a major furnish for fine quality writing, printing, and other paper grades. More than 9 million tons of straw pulp is produced annually in China, which accounts for about 90% of the world's total straw pulp [6]. However, in developed countries, the straw fibers are used in the manufacturing of a variety of high-value specialty papers (such as filter paper, bank-notes paper, security paper, or condenser papers). Unfortunately, these straws and all Gramineae are covered on both culm and leaf with semicrystalline wax particles, 1–2  $\mu\text{m}$  across and 2–3  $\mu\text{m}$  high. These waxes are mainly long-chain alkane, ester, and alcohol waxes together with noticeable amounts of free fatty acids [7]. Furthermore, published studies on the chemical composition of extractives from straw refer mainly to the more polar fraction containing phenolics compounds [8]. To the best of our knowledge, extractives are formed through a variety of biosynthetic pathways [9], which is illustrated for terpenes in Fig. 3.1. Recently, there have been many studies published concerning the composition of extractives from some hardwood and softwood commonly used in the pulp and paper industry. However, there has been little investigation into the quantitative characterization of straw lipophilic extractives to date. In this chapter, the isolation and purification of extractives from the straw and their structural characterization are reviewed.

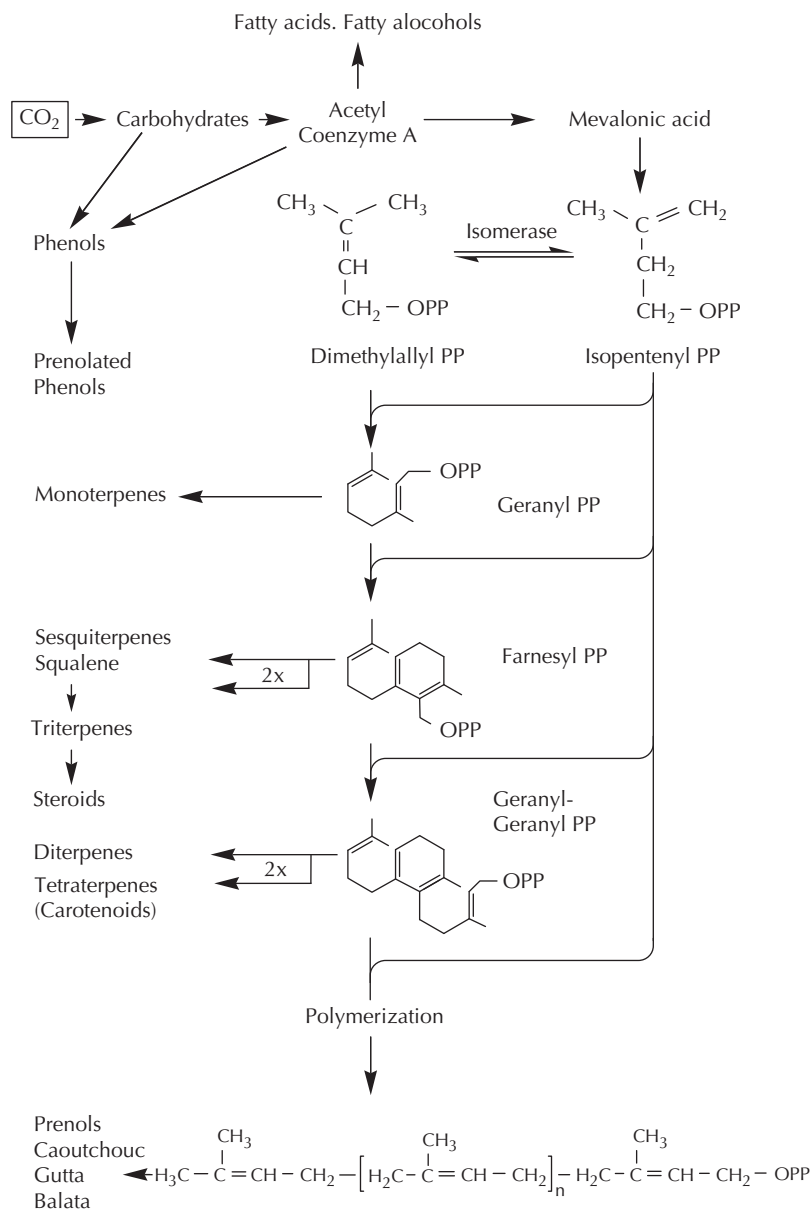


FIGURE 3.1 Biosynthesis of terpenes. PP, pyrophosphate [9].

### 3.1 ISOLATION AND PURIFICATION

Conventional approaches used to reduce wood extract deposits include the use of wood species that yield low in extracts and the debarking or seasoning of logs. During seasoning, extracts undergo volatilization, enzymatic hydrolysis, and air oxidation. Unfortunately, these reactions are slow, particularly in cold weather. Furthermore, a long period of seasoning inflates costs and promotes microbial deterioration [2]. Recently, new biological methods using fungal pretreatment have been developed to reduce extract levels with a minimal period of storage. However, the fungi are poorly adapted to low temperatures and to some of the wood species used, and some fungi appear effective only on certain

wood types or straws and under specific pulping conditions [5]. Efforts to find a solution to this problem must begin with the development of a rapid, simple, and quantitative method for separating and identifying the complex lipid extracts in wood and straw samples. Such a method would be useful in understanding pitch formation and optimizing reactions to remove the extracts prior to pulping.

#### 3.1.1 Procedures for Isolation of Extractives

Traditional methods for the extraction of extractives in pulp and paper matrices usually use solvents, such as benzene, dichloromethane, and chloroform. The mass of substances

extracted from fresh wood or straw samples decreased as the polarity of solvents decreased in the order, methanol > acetone > dichloromethane > hexane. However, the relative amounts of individual components in the lipophilic extracts were not substantially different from various extraction solvents [10]. Additionally, no single solvent is capable of removing all the lipophilic substances, and different solvents remove different combinations of lipids [11]. Several workers [12–14] have found acetone to be a suitable solvent for the analysis of extractives. Perhaps this solvent may be adopted as a standard for the determination of the extractives. Modern procedures to isolate the extractives include rapid extraction techniques such as supercritical fluid extraction (SFE) and Soxhlet extraction [1].

#### 3.1.1.1 Supercritical Fluid Extraction

A supercritical fluid is a substance for which the temperature and the pressure are simultaneously above its critical point. A fluid in the supercritical state has the penetration and transport properties approaching those of a gas but acts like a liquid when dissolving analytes from a matrix [15]. A small change in the pressure of a supercritical fluid results in a huge change in its density, and the solvent strength of the fluid changes with varying density. SFE has more advantages [15] than liquid solvent extractions. First, SFE is a rapid process for such quantitative extractions, which can be accomplished between 10 and 60 min, whereas the time of liquid solvent extraction varies from several hours to even a few days. Second, the solvent strength of a supercritical fluid can be easily controlled. At a constant temperature, extraction at lower pressures will favor less polar analytes. In contrast, extraction at higher pressures will favor more polar and higher molar mass analytes. This enables an extraction to be optimized for a particular compound class by simply changing the pressure of the extraction. Thus, class-selective extractions can be performed by extracting a single sample at different pressures. Third, most supercritical fluids are relatively inert, pure, nontoxic, and inexpensive. The generation of liquid waste solvents and exposure of laboratory personnel to hazardous solvents can be reduced or eliminated. By this token, SFE is very new and efficient to isolate the extractives. Demirbas [14] found that supercritical extraction of beech wood with acetone yielded values that were four times higher than the values yielded by the Soxhlet acetone extraction for the same wood. On the other hand, recoveries could be improved by adding an organic modifier (1% acetic acid in methanol) to the supercritical fluid [16].

#### 3.1.1.2 Soxhlet Extractions

The traditional solvent extraction (e.g., with acetone, chloroform, dichloromethane, etc.) is routinely used to determine the wood resin or extractives content in samples

of straw, wood, pulp, paper, or pitch deposits. Traditionally, this has been carried out in a Soxhlet extractor. Sun et al. [17–22] chose a different solvent such as toluene ethanol (2/1, v/v), chloroform, petroleum ether, dichloromethane, hexane, and methyl *tert*-butyl ether (MTBE) to test their efficacy for the extraction of straw (wheat, barley, rye, and rice) samples for 12 h in a Soxhlet apparatus. After the solvent removal with a rotary vacuum evaporator at 35 °C, the mixture was further dried in the nitrogen steam and then weighed to determine the extract yield. In addition, their chemical compounds were determined by gas chromatography (GC) and high-performance liquid chromatography (HPLC).

### 3.1.2 Purification of Extractives

#### 3.1.2.1 Solid-phase Extraction

Traditional liquid solid extraction involves the mixture of a surface active solid adsorbent with a sample solution. This process leads to the sample components to be partitioned between the liquid and solid phases. One of the principal advantages of liquid solid extraction is that, unlike liquid liquid extraction, by choosing suitable selective adsorbents, the partition equilibrium of specific sample components can be driven to favor the nearly complete adsorption or desorption [23]. Solid-phase extraction (SPE) is ideally suited for the sample preparation of diverse compounds such as extractives in deposits, wood resin in pulp, additives, and organics in process streams. Judicious choice of solid-phase adsorbent, solvent, sample pretreatment, and postcolumn analytical techniques can minimize or even eliminate the emulsion problems. Moreover, these properties of consuming lower amounts of samples, less solvent volume, and more rapid purification of sample make SPE superior to the traditional extraction procedures [1].

For a better characterization of the different homologous series, the lipid extracts were purified by a SPE procedure. Gutiérrez et al. [4] demonstrated the application of SPE techniques to the lipophilic extractives from *Eucalyptus globules* Labill. The procedure used 500 mg of aminopropyl-phase cartridges from Waters (a division of Millipore), as described earlier [5]. Briefly, the lipophilic extractives were obtained by redissolving the dried acetone extract in chloroform and evaporating under nitrogen to dryness. The dried chloroform extracts were taken up in a minimal volume (<0.5 ml) of hexane:chloroform (4:1) and loaded into the cartridge column previously conditioned with hexane (4 ml). The cartridge was loaded and eluted by gravity. The column was first eluted with 8 ml of hexane and subsequently with 6 ml of hexane:chloroform (5:1), with 10 ml of chloroform and finally with 10 ml of diethyl ether:acetic acid (98:2). Each isolated fraction was dried under nitrogen and analyzed by GC and GC/MS.



## 3.2 STRUCTURAL CHARACTERIZATION

Classical methods for quantitatively analyzing extracts are time consuming. Such methods involve separating free fatty acids and resin acids from the neutral materials by ion-exchange chromatography, saponifying the neutral fraction, and finally, methylating the acid fractions prior to analysis by gas chromatography (GC). Since 1970, the speed of techniques has simplified the analyses of extracts. Thin layer chromatography (TLC) has been widely used for the separation of various extracts, but no TLC separation that allows the simultaneous separation and quantification of the fatty acids, resin acids, and triglycerides in a single analysis has been reported [24]. HPLC has been adapted to the analysis of the total extracts in softwoods [24] and resin acids in aqueous samples [25]. The related technique, high-performance size-exclusion chromatography, has allowed the separation of extracts from effluents of softwood processing [26], whereas the high-performance TLC has provided a convenient method for analysis of resin in wood, pulp, and paper [27]. Gel permeation chromatography has also been used for the separation and semiquantitative analysis of wood extracts [28]. Other approaches to the analysis involve Fourier transform infrared (FTIR) [29] and  $^{13}\text{C}$ -nuclear magnetic resonance ( $^{13}\text{C}$ -NMR) spectroscopy studied from wood resin and pitch sample [30–32]. Particularly in the past 10 years, the chemical characterization of the lipophilic extractives has been performed by GC and gas chromatography and mass spectrometry (GC/MS) by using high-temperature short- and medium-length capillary columns [33], respectively. This method enables the elution and analysis of those extractives.

The extractives have been silylated [20] before analysis by GC/MS. The dried lipophilic extracts (approximately 3 mg) from straw were silylated with 120- $\mu\text{l}$  bis-trimethylsilyl-trifluoroacetamide and 60- $\mu\text{l}$  trimethylchlorosilane. The reactions were completed by keeping the glass-stoppered test tubes in an oven at 75 °C for 20 min. After cooling, 180- $\mu\text{l}$  toluene was added. The solution was shaken and was ready for analysis by GC. The silylated sample was analyzed by GC on an Rtx-1 capillary column coated with cross-linked 100% dimethyl polysiloxane (15 m, 0.53 mm i.d., 0.10  $\mu\text{m}$  film thickness, purchased from Hewlett-Packard Company, Beijing) with a flame ionization detector (FID). Helium was used as the carrier gas. The injector and detector temperatures were held at 340 °C. The oven was temperature-programmed from 70 °C to 340 °C (2 min) at 8 °C/min. A sample of solution (1  $\mu\text{l}$ ) was injected in splitless mode and changed to split mode after 0.5 min. The total analysis time including the cooling of the column oven and injector, followed by temperature stabilization, was about 45 min. Each compound of free fatty acids, resin acids, and sterols was identified both by comparison of their gas chromatographic retention times and by total ion detection mass spectra with those in authentic

compounds. Individual components of the waxes, sterol esters, and triglycerides were verified only by GC retention times because GC-MS yielded only fragments by electron-impact MS and rarely yielded detectable molecular ions [4]. On the other hand, previous studies found that the major components of sterol esters in the wheat straw were  $\beta$ -sitosterol esters [34]. However, these compounds were not available commercially and were, therefore, replaced by the closely related compounds, cholesteryl esters, as standards in the study. The FID response was assumed to be same for the component group corresponding to each class of standards used.

The phenolic compounds in the extractives were quantitatively analyzed by HPLC on a Spherisorb 50 DS column of dimensions 250  $\times$  4.6 mm (Phenomenex). Samples (approximately 10 mg) were dissolved in 1 ml methanol, and separation were obtained using a linear gradient of two solvent systems: solvent A (water methanol acid, 89:10:1) and solvent B (methanol water acetic acid, 90:9:1). A linear gradient was run over 31 min from 0 to 40% B at a flow rate of 1 ml/min. The compounds were detected at 280 nm and calculated to each of the standards using Kontron MT 450 software. Calibration curves were established with appropriate mixtures of authentic phenolic acids and aldehydes. The lignans (phenyl propane dimers) were quantitatively estimated based on results from calibration runs of a reference mixture containing 2,2'-dihydroxybiphenyl as biphenyl model compound [21].

### 3.2.1 Chemical Composition of Extractives

The content of extractives and their composition vary greatly among different straw species and producing area. The straw extractives can be divided into two subgroups: lipophilic and phenolic compounds.

#### 3.2.1.1 Lipophilic Compounds

The chemical composition of seven lipophilic extractives from wheat straw by extraction with toluene ethanol (2:1, v/v), chloroform methanol (2:1, v/v), methyl *tert*-butyl ether, hexane, petroleum ether, dichloromethane, and toluene ethanol methanol (1/1/1, v/v/v) for 6 h in a Soxhlet apparatus, respectively, and the water-soluble lipophilic extract has been examined at 98 °C for 4 h by Sun et al. [17, 21]. As shown in Table 3.1, the extractions with toluene ethanol or chloroform methanol and/or toluene ethanol methanol yielded larger amounts of extractives (2.38% dry straw for F1, 2.32% for F2, 2.66% for F8). The yields of MTBE (F3), dichloromethane (F6), and water (F7) extracts from the straw accounted for 1.00, 1.17, and 0.91%, respectively. However, extraction with hexane (F4) or petroleum ether (F5) yielded lower amounts of extractives

**TABLE 3.1** Yield (% Dry Straw) and Chemical Composition (% Dry Extractives) of Extractives in Wheat Straw [17, 21]

Yield/composition	Peak no.	Extraction							
		F1	F2	F3	F4	F5	F6	F7	F8
Yield (% dry straw)		2.38	2.32	1.00	0.74	0.55	1.17	0.91	2.66
Chemical composition									
Free fatty acids	P1 27	32.3	28.2	26.6	19.1	15.4	11.6	33.8	40.3
All compounds identified		31.7	26.2	23.3	15.5	11.6	10.9	23.6	39.6
Decanoic acid (C10:0)	P1	0.1	0.1	0.2	0.1	0.0	0.1	0.9	0.1
Dodecanoic acid (C12:0)	P3	0.4	0.2	0.2	0.1	0.2	0.2	1.2	0.2
Tetradecanoic acid (C14:0)	P5	13.7	10.8	5.2	2.0	1.4	1.3	1.4	14.4
Pentadecanoic acid (C15:0)	P6	10.4	8.0	5.3	1.1	1.1	1.1	8.9	11.0
Hexadecenoic acid (C16:1)	P8	0.3	0.9	1.7	1.0	0.3	0.6	0.9	0.6
Hexadecanoic acid (C16:0)	P9	4.6	4.5	6.2	5.4	3.2	3.9	5.5	7.0
Heptadecanoic acid (C17:0)	P11	0.3	0.1	0.5	0.7	0.4	0.2	0.2	0.5
Linoleic acid (C18:2) + oleic acid (C18:1)	P12	0.5	0.8	2.1	2.9	3.3	2.0	1.3	1.3
Octadecanoic acid (C18:0)	P13	0.0	0.1	0.5	0.5	0.4	0.3	0.3	0.2
Nonadecanoic acid (C19:0)	P15	0.1	0.1	N	N	N	N	0.1	0.3
Gondoic acid (C20:1)	P16	0.0	0.0	0.2	0.1	0.1	0.1	0.1	0.1
Eicosanoic acid (C20:0)	P17	0.2	0.1	0.4	0.5	0.3	0.3	0.4	2.4
Heneicosanoic acid (C21:0)	P19	0.0	0.1	0.1	0.1	0.1	0.1	1.2	0.1
Docosanoic acid (C22:0)	P21	0.2	0.1	0.4	0.6	0.4	0.4	0.7	0.2
Tetracosanoic acid (C24:0)	P25	0.8	0.4	0.4	0.4	0.4	0.3	0.5	1.2
Resin/other acids	P4,7,14	23.4	20.1	5.1	4.3	4.2	2.7	5.5	22.2
All compounds identified		23.4	20.1	5.1	4.3	4.2	2.7	5.5	22.2
Azelaic acid	P4	16.2	15.2	2.8	1.8	1.9	1.2	3.9	13.8
Maleic acid	P7	7.1	4.7	2.1	2.3	1.6	1.4	1.5	7.9
Abietic acid	P14	0.1	0.2	0.2	0.2	0.7	0.1	0.1	0.5
Sterols	P28 31	4.1	4.3	15.5	24.0	24.5	17.8	6.1	3.2
All compounds identified		4.1	4.3	15.5	24.0	24.5	17.8	6.1	3.2
Cholesterol	P28	0.2	0.2	1.5	2.3	2.1	1.1	0.5	0.6
Ergosterol + stigmasterol + $\beta$ sitosterol	P29,30	2.9	3.4	13.9	20.7	21.5	16.2	4.3	2.6
Stigmastanol	P31	1.0	0.7	0.1	1.0	0.9	0.5	1.3	
Waxes	P32 38	1.4	2.6	17.0	21.1	20.8	15.8	8.2	2.2
All compounds identified		1.3	2.1	10.7	14.1	14.1	10.9	5.9	1.9
Palmityl palmitate	P32	0.6	1.0	4.7	8.1	7.6	4.6	2.5	0.7
Oleyl palmitate	P35	0.6	1.0	5.9	5.8	6.3	6.2	3.3	1.2
Oleyl oleate	P37	0.1	0.1	0.1	0.2	0.2	0.1	0.1	N

(Continued)

**TABLE 3.1** Yield (% Dry Straw) and Chemical Composition (% Dry Extractives) of Extractives in Wheat Straw [17, 21]—cont'd

Yield/composition	Peak no.	Extraction							
		F1	F2	F3	F4	F5	F6	F7	F8
Diglycerides	P39,40	0.8	0.5	0.2	0.2	0.4	0.4	0.2	0.1
All compounds identified		0.4	0.3	0.1	0.1	0.2	0.2	0.1	0.1
Dipalmitin	P39	0.4	0.3	0.1	0.1	0.2	0.2	0.1	0.1
Steryl esters (mainly sitosteryl esters)	P41 50	13.9	10.4	11.3	12.5	12.6	19.1	6.8	3.2
All compounds identified		8.7	7.6	8.0	10.2	10.2	15.2	4.3	2.7
Steryl laurate	P43	3.2	1.0	0.6	0.9	1.1	0.6	1.9	0.4
Steryl mysistate	P45	2.6	2.5	2.5	2.7	2.9	4.9	1.4	1.2
Steryl palmitate	P47	1.4	1.4	1.3	2.2	2.2	4.5	0.5	0.5
Steryl heptadecanoate	P48	1.0	1.4	2.2	2.0	1.4	3.4	0.3	0.3
Steryl oleate	P49	0.5	1.3	1.4	2.4	2.6	1.8	0.2	0.3
Triglycerides	P51 59	1.7	8.9	9.7	13.4	14.2	12.5	3.5	2.3
All compounds identified		1.3	4.0	2.7	5.3	8.1	6.9	2.6	1.5
Tripalmitin	P52	0.5	0.8	0.1	0.7	0.8	0.5	0.5	0.6
Dipalmitoyl oleoylglycerol	P53	0.4	1.5	1.0	1.8	3.0	2.8	1.0	0.3
Triolein	P56	0.4	1.7	1.6	2.8	4.3	3.6	1.1	0.6
Total lipophilic substances		54.2	54.9	80.3	90.3	87.9	77.2	58.6	71.1
Total substances		77.6	75.0	85.4	94.6	92.1	79.9	64.1	73.5

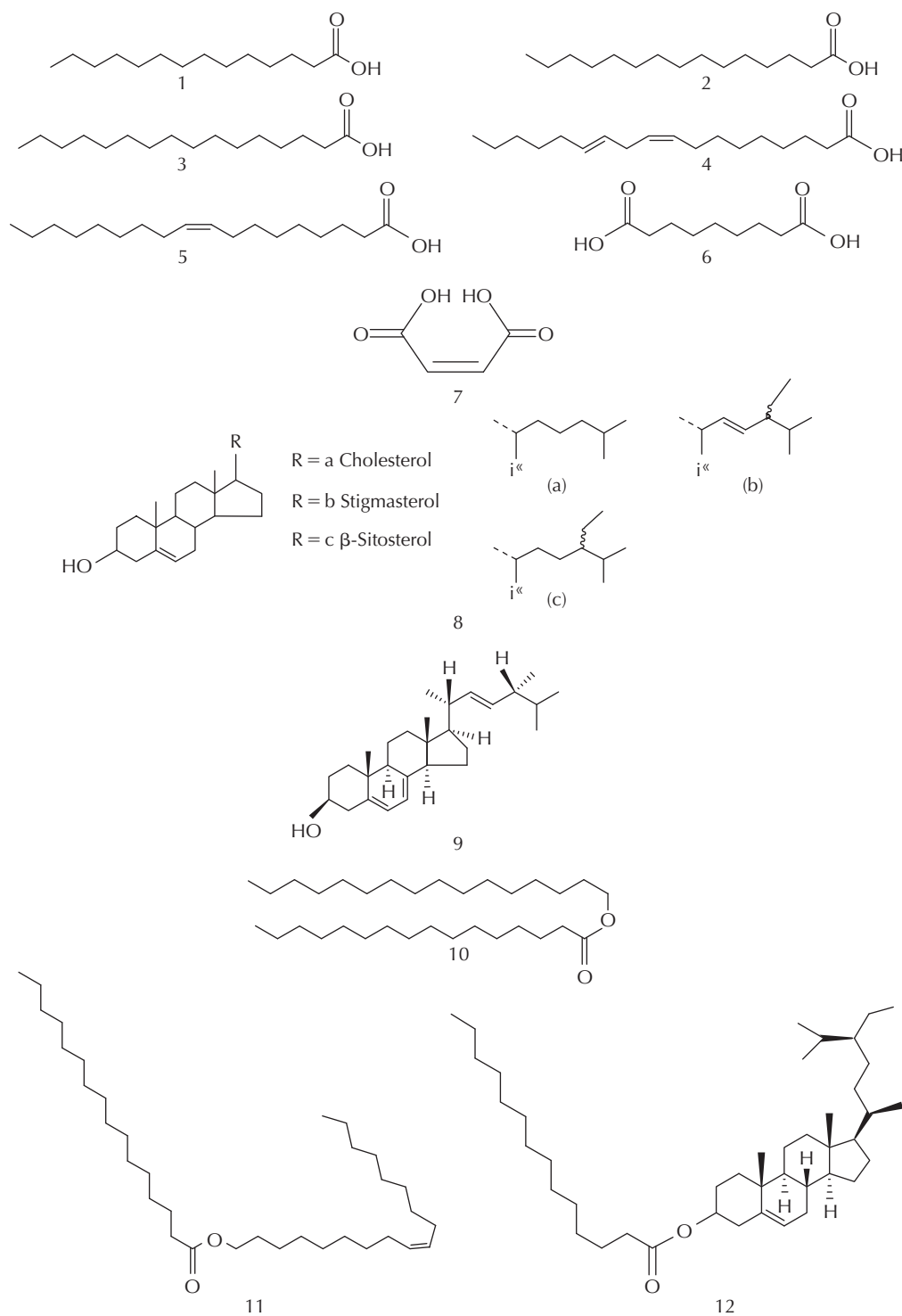
Fractions of the extractives obtained by extraction with toluene–ethanol (2:1, v/v; F1), chloroform–methanol (2:1, v/v; F2), methyl tert-butyl ether (F3), hexane (F4), petroleum ether (F5), dichloromethane (F6), and toluene–ethanol–methanol (1/1/1, v/v/v; F8) for 6 h in a Soxhlet apparatus, respectively. F7 represents the extractive fraction solubilized during the treatment of wheat straw with water at 98 °C for 4 h.

N indicates not detectable.

(0.74% for F4, 0.55% for F5). There is no one method to extract all the lipophilic substances with one kind of organic solvent, and different solvents could remove different combinations. The purity of lipophilic substances in the extracts obtained by MTBE, hexane, petroleum ether, dichloromethane, and toluene ethanol methanol accounted for 80.3, 90.3, 87.9, 77.2, and 71.1%, respectively, which indicated the yields were higher than those in the extracts obtained by toluene ethanol (54.2%), chloroform methanol (54.9%), and water (58.6%). This indicated that the latter two mixtures of solvents and water extracted some nonlipophilic materials that are not analyzed by the GC system except for the azelaic and maleic acids. The hexane and petroleum ether yielded high percentages of lipophilic extractives but did not extract significant amounts of azelaic or maleic acid. If only lipophilic extractives are of interest, hexane and petroleum ether are good solvents, yielding extracts containing 88–90% lipophilic extractives. MTBE and dichloromethane extracts yielded similar values

(77–80%). This particularly reflected in the amounts of sterols and waxes (4.3 and 2.6%, respectively) in the chloroform methanol extract and 24.0 and 21.1%, respectively. However, if the purity of the lipophilic extractives is considered, MTBE and dichloromethane could be useful solvents for the extraction of wheat straw, although they did not have much more yield of lipophilic extractives. Five main lipid classes (free fatty acids/resin acids, sterols, waxes, steryl esters, triglycerides) were identified, and their individual components were quantified by GC as their tetramethyl silicane (TMS) ester (free fatty acids/resin acids) and TMS ethers (sterols). The structures of main lipophilic compounds in wheat straw are shown in Fig. 3.2.

The chromatogram of the total lipophilic extract, obtained by extraction with MTBE from wheat straw, is shown in Fig. 3.3, and the compositions of all the compounds identified quantitatively are listed in Table 3.1 [17]. Obviously, the extractives in wheat straw comprise a large number of components. Free fatty acids (11.6–40.3%),

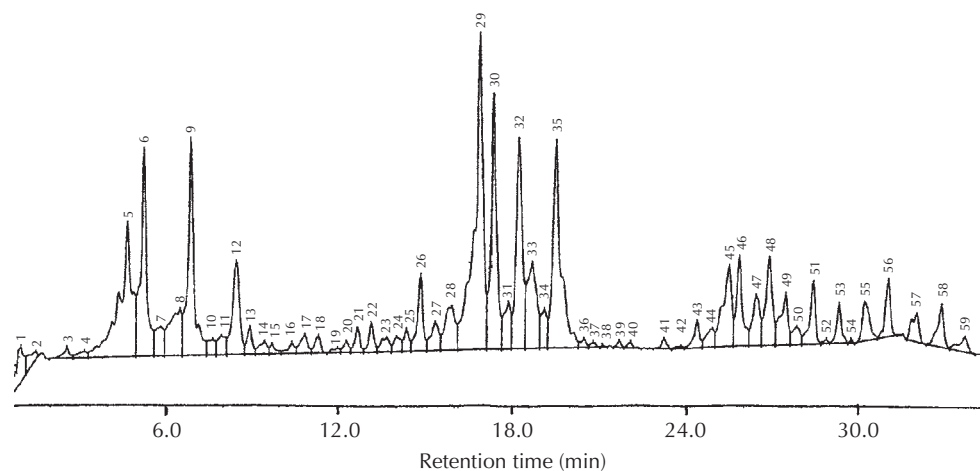


**FIGURE 3.2** Structures of the main lipophilic compounds present in wheat straw. 1. Tetradecanoic acid; 2. Pentadecanoic acid; 3. Hexadecanoic acid; 4. Linoleic acid; 5. Oleic acid; 6. Azelaic acid; 7. Maleic acid; 8. Cholesterol (a), Stigmasterol (b),  $\beta$ -Sitosterol (c); 9. Ergosterol; 10. Palmityl palmitate; 11. Oleyl palmitate; 12. Steryl mysistate [17].

sterols (3.2 24.5%), waxes (1.4 21.1%), sterol esters (3.2 19.1%), and triglycerides (1.7 14.2%) were the major lipid groups present in the extracts. The GC results showed a relatively high concentration of free fatty acids in

all the lipophilic extractives from wheat straw. Their distributions were identified in the range from C10 to C24. Tetradecanoic acid (C14:0), pentadecanoic acid (C15:0), hexadecanoic acid (C16:0), linoleic acid (C18:2),





**FIGURE 3.3** Chromatogram of lipophilic extractives obtained by a Soxhlet extraction with methyl tert butyl ether from wheat straw [17].

and oleic acid (C18:1) were the major components. It is interesting to note that the extracts contained 1.2 16.2% azelaic acid and 1.4 7.9% maleic acid. They were particularly high in the extracts obtained with toluene ethanol (F1) or chloroform methanol (F2), which yielded 15.2 16.2% azelaic acid and 4.7 7.1% maleic acid. Chaves das Neves and Gaspar [34] reported that wheat straw extractives and maleic acid appeared in a noticeable amount.

Sterols were the second most important class of compounds in wheat straw extractives, accounting for 15.5, 24.0, 24.5, and 17.8% in F3, F4, F5, and F6 extracts, respectively.  $\beta$ -Sitosterol was the main sterol in the extracts. Cholesterol, ergosterol, stigmasterol, and stigmastanol were also identified from the extracts but in small amounts.

Waxes were another major class of lipids in wheat straw extractives, comprising 15.8 21.1% of the total F3 F6 extracts. However, the extracts obtained with toluene ethanol (F1), chloroform methanol (F2), and toluene ethanol methanol (F8) yielded only 1.4, 2.6, and 2.2% of waxes, respectively. Palmityl palmitate and oleyl palmitate were found to be the major compounds and accounted for 62.4 68.4% of the total waxes in the above four extracts (F3 F6) as revealed by GC.

Steryl esters were also important constituents of the lipophilic extractives in wheat straw, accounting for 3.2 19.1% of the extracts. During characterization of the composition of steryl esters, it was impossible to identify individual sterol ester by GC-MS, as they only show fragments arising from the sterol moiety by electron-impact MS and rarely give detectable molecular ions. A similar phenomenon was observed in the GC-MS analyses for triglycerides. In our study, these two classes of lipids were, therefore, identified only by comparing retention times with authentic compounds of GC. To correct the analyses, the individual peak area of steryl esters and triglycerides was divided by 0.74 and 0.60, respectively. The steryl esters consisted of the combinations of the major fatty acids (lauric, myristic, palmitic, heptadecanoic, and oleic acids) with sterols

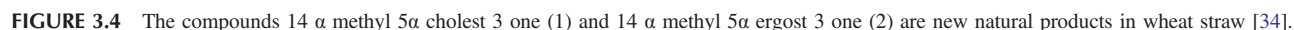
(mainly  $\beta$ -sitosterol). The distribution of esterified fatty acids was the same as that of the free fatty acids, as described above. Steryl laurate, steryl mysistate, steryl palmitate, steryl heptadecanoate, and steryl oleate together comprised 62.5 84.4% of the total steryl esters in eight lipophilic extractives.

Finally, the triglycerides identified among the straw extractives accounted for 8.9 14.2% in F2 F6 fractions and only 1.7, 3.5, and 2.3% in toluene ethanol, water, and toluene ethanol methanol, respectively. These triacylglycerols are commonly produced as energy reserves and carbon skeletons for growth and development, as they are twice as efficient for metabolic energy as either proteins or carbohydrates.

Diacylglycerols are present in trace amounts in fresh animal and plant tissues and may be intermediates in the synthesis of triacylglycerols. In the present study, diglycerides were also identified among the lipophilic extractives from wheat straw, although in small amounts (0.1 0.8%). Dipalmitoyl glycerol was the only single compound identified.

Besides the above composition of extractives from wheat straw, two previously unknown sterolic compounds were detected by Chaves das Neves and Gaspar [34], and the analysis by HPLC-MS revealed the presence of two new ketosterols. The sterolic compounds were extracted with acetone water (2.8:1.2; 80 litre) for 24 h from wheat straw and then were purified. The sterolic fraction was further studied by HPLC-MS. Among the 37 components identified, two new ketosterols named 14- $\alpha$ -methyl-5 $\alpha$ -cholest-3-one and (24*R*)-14 $\alpha$ -methyl-5 $\alpha$ -esgost-3-one were characterized by means of their MS spectra. The structures are shown in Fig. 3.4.

Table 3.2 shows extractives from rice straw by extraction with toluene ethanol (2:1, v/v), chloroform, petroleum ether, dichloromethane, hexane, and methyl-*tert*-butyl ether for 12 h in a Soxhlet apparatus by Sun et al. [18, 20]. Obviously, a mixture of toluene ethanol produced highest yield (3.42%), and petroleum ether and hexane



Yield/composition	Peak no.	Extractives					
		F1 <sup>a</sup>	F2 <sup>a</sup>	F3 <sup>a</sup>	F4 <sup>a</sup>	F5 <sup>a</sup>	F6 <sup>a</sup>
Yield (% dry straw)		3.42	1.19	0.45	1.37	0.65	0.97
Chemical composition							
Free fatty acids/resin/other acids	P1 24	29.27	19.42	26.77	23.66	18.26	20.30
All compounds identified		28.96	16.86	24.29	17.51	16.71	15.98
Decanoic acid (C10:0)	P1	0.28	0.15	0.16	0.12	0.11	0.67
Dodecanoic acid (C12:0)	P3	0.49	0.15	0.71	0.15	0.12	0.16
Azelaic acid	P5	3.98	1.45	1.01	0.69	0.66	0.62
Tetradecanoic acid (C14:0)	P6	4.88	0.47	0.87	0.28	0.24	3.62
Pentadecanoic acid (C15:0)	P7	3.02	0.36	0.73	1.28	0.38	0.57
Maleic acid	P8	6.47	0.89	1.68	0.58	1.19	0.52
Hexadecenoic acid (C16:1)	P9	1.94	1.38	2.96	1.36	2.08	0.42
Hexadecanoic acid (C16:0)	P10	3.82	5.01	8.11	4.18	4.65	3.96
Heptadecanoic acid (C17:0)	P11	0.86	1.05	1.38	1.50	1.15	0.37
Linoleic acid (C18:2) + oleic acid (C18:1)	P12	1.22	2.18	3.35	2.41	2.52	2.09
Octadecanoic acid (C18:0)	P13	0.23	0.49	0.68	0.78	0.61	0.42
Abietic acid	P14	0.25	0.52	0.59	1.21	0.16	0.12
Nonadecanoic acid (C19:0)	P15	0.16	0.18	0.26	0.53	N	N
Gondoic acid (C20:1)	P16	N	N	N	N	N	0.21
Eicosanoic acid (C20:0)	P17	0.19	1.15	1.25	1.08	1.12	0.60
Heneicosanoic acid (C21:0)	P18	0.39	0.18	0.24	0.19	0.21	0.18
Docosanoic acid (C22:0)	P20	0.29	0.47	0.72	0.49	0.70	0.47
Tetracosanoic acid (C24:0)	P22	0.49	0.78	0.97	0.68	0.84	0.98
Sterols	P25 28	4.10	10.50	28.48	8.98	19.83	14.60
All compounds identified		4.10	10.40	28.48	8.98	19.83	14.60
Cholesterol	P25	0.24	0.27	0.57	0.27	1.23	0.32
Ergosterol + stigmasterol + β sitosterol	P26 28	3.86	10.23	27.91	8.61	18.60	14.28

(Continued)

**TABLE 3.2** The Yield (% Dry Straw) and Chemical Composition (% Dry Extractives) of Extractives in Rice Straw [18]—cont'd

Yield/composition	Peak no.	Extractives					
		F1 <sup>a</sup>	F2 <sup>a</sup>	F3 <sup>a</sup>	F4 <sup>a</sup>	F5 <sup>a</sup>	F6 <sup>a</sup>
Waxes	P29 37	4.68	8.63	14.01	6.58	12.62	9.53
All compounds identified		2.06	3.20	6.13	2.38	3.47	4.34
Palmityl palmitate	P30	1.55	2.48	4.82	1.65	2.28	3.04
Oleyl palmitate	P33	0.35	0.52	1.08	0.45	0.81	0.96
Oleyl oleate	P35	0.16	0.20	0.23	0.28	0.38	0.34
Diglycerides	P38 39	0.23	0.19	0.30	0.33	0.32	0.42
All compounds identified		0.14	0.11	0.19	0.18	0.17	0.22
Dipalmitin	P38	0.14	0.11	0.19	0.18	0.17	0.22
Steryl esters (mainly sitosteryl esters)	P40 48	6.35	12.44	6.26	12.38	6.45	18.19
All compounds identified		3.69	8.79	4.88	8.58	5.01	10.83
Steryl laurate	P41	0.46	0.28	0.42	0.29	0.32	0.29
Steryl mysistate	P43	1.26	2.82	0.72	2.26	0.58	3.20
Steryl palmitate	P45	0.64	1.84	1.76	1.95	1.58	2.28
Steryl heptadecanoate	P46	0.76	2.21	0.59	2.35	0.92	2.93
Steryl oleate	P48	0.57	1.64	1.39	1.73	1.61	2.13
Triglycerides	P49 63	5.59	10.20	11.31	8.59	14.83	11.38
All compounds identified		2.45	4.91	3.94	3.51	3.62	5.33
Tripalmitin	P50	0.66	1.14	0.29	0.78	0.35	1.64
Dipalmitoyl oleoylglycerol	P52	0.59	0.98	0.42	0.41	0.48	2.08
Triolein	P55	1.20	2.79	3.23	2.32	2.79	1.61
Total lipophilic substances		39.77	59.04	84.44	59.25	70.45	73.28
Total substances		50.22	61.38	87.13	60.52	72.30	74.42

<sup>a</sup>Fractions of the extractives obtained by extraction with toluene–ethanol (2:1, v/v; F1), chloroform (F2), petroleum ether (F3), dichloromethane (F4), hexane (F5), and methyl-tert-butyl ether (F6) for 12 h in a Soxhlet apparatus from rice straw.

N indicates not detectable.

yielded approximately equal, lowest amounts of extractives (0.45%, 0.65%), whereas chloroform, dichloromethane, and MTBE yielded medium values (1.19, 1.37, 0.97%). As shown in table, free acids (14.72–24.08%), sterols (4.10–28.48%), waxes (4.68–14.01%), steryl esters (6.35–18.19%), and triglycerides (5.59–14.83%) were the major five lipid groups present in the rice straw lipophilic extractives.

Table 3.3 shows extractives from barley straw by extraction with toluene ethanol (2:1, v/v), chloroform, MTBE, hexane acetone (2:1, v/v), and dichloromethane and from rye straw by extraction with MTBE for 12 h in a Soxhlet apparatus by Sun et al. [19, 20]. For barley straw,

extraction with MTBE produced the lowest yield of total extracts (1.19%) but contained the highest amounts of lipophilic extracts (81.04%). The chemical compositions are similar with wheat and rice straw; the major five lipid groups are free fatty acids (15.18–38.20%), sterols (1.41–10.42%), waxes (3.45–11.87%), steryl esters (5.04–24.70%), and triglycerides (1.16–10.03%).

In addition, Sun et al. [35] also reported the chemical composition and characterization of hot water-soluble lipophilic extractives from wheat straw. As shown in Table 3.4, it was found that treatment of wheat straw with hot water at 80–95 °C for 0.5 h at pH 6.0–8.0 released 41.0–53.0% of the original lipophilic extractives. The

**TABLE 3.3** The Yield (% Dry Straw) and Chemical Composition (% Dry) of Barley and Rye Straw Extracts [19]

Yield/composition	Peak no.	Barley straw extractives					Rye straw extractives
		F1 <sup>a</sup>	F2 <sup>a</sup>	F3 <sup>a</sup>	F4 <sup>a</sup>	F5 <sup>a</sup>	F6 <sup>a</sup>
Yield (% dry straw)		3.92	1.21	1.19	2.10	1.65	1.01
Chemical composition							
Free fatty acids/resin/other acids	P1 24	53.62	15.78	24.77	33.73	21.09	23.65
All compounds identified		52.03	12.02	19.86	23.71	16.01	14.22
Decanoic acid (C10:0)	P1	0.23	0.15	0.16	0.14	0.11	0.37
Dodecanoic acid (C12:0)	P3	0.80	0.20	0.18	0.42	0.60	0.21
Azelaic acid	P5	7.98	0.48	0.75	3.08	1.48	0.65
Tetradecanoic acid (C14:0)	P6	13.84	3.31	7.50	4.86	4.65	2.95
Pentadecanoic acid (C15:0)	P7	9.43	1.26	1.99	2.95	1.65	0.39
Maleic acid		7.08	N	N	N	N	N
Hexadecenoic acid (C16:1)	P8	1.56	0.82	0.93	0.86	0.78	N
Hexadecanoic acid (C16:0)	P9	7.96	2.55	3.31	4.25	1.88	4.24
Heptadecanoic acid (C17:0)	P10	0.11	0.22	0.73	0.55	0.51	0.25
Linoleic acid (C18:2) + oleic acid (C18:1)	P11	0.16	1.78	2.60	3.23	2.53	1.87
Octadecanoic acid (C18:0)	P12	0.58	0.11	0.28	0.42	0.18	0.30
Abietic acid	P14	0.36	0.12	0.42	0.57	0.24	0.05
Nonadecanoic acid (C19:0)		0.12	N	N	0.30	0.11	N
Gondoic acid (C20:1)	P15	0.13	0.15	0.07	0.30	0.06	0.18
Eicosanoic acid (C20:0)	P16	0.29	0.40	0.30	0.45	0.32	0.70
Heneicosanoic acid (C21:0)	P17	0.21	0.06	N	0.10	0.10	0.20
Docosanoic acid (C22:0)	P18	0.51	0.18	0.38	0.46	0.43	0.66
Tetracosanoic acid (C24:0)	P22	0.68	0.23	0.26	0.78	0.41	1.20
Sterols	P25 28	1.41	7.11	10.42	6.20	8.40	12.54
All compounds identified		1.41	7.11	10.42	6.20	8.40	12.54
Cholesterol	P25	0.48	0.18	0.52	0.35	0.47	0.84
Ergosterol + stigmasterol + $\beta$ sitosterol	P26 28	0.93	6.93	9.90	5.85	7.93	11.70
Waxes	P29 32	3.45	11.18	11.22	8.54	11.87	27.14
All compounds identified		3.11	7.71	8.41	6.13	7.53	15.06
Palmityl palmitate	P29	0.62	4.69	6.36	3.95	4.55	10.51
Oleyl palmitate	P31	2.49	3.02	2.05	2.18	2.98	4.39
Oleyl oleate	P32	N	N	0.36	N	N	0.16
Diglycerides	P33,34	0.07	0.57	0.65	0.38	0.89	0.23
All compounds identified		0.05	0.29	0.34	0.24	0.51	0.05
Dipalmitin	P33	0.05	0.29	0.34	0.24	0.51	0.05

(Continued)



**TABLE 3.3** The Yield (% Dry Straw) and Chemical Composition (% Dry) of Barley and Rye Straw Extracts [19]—cont'd

Yield/composition	Peak no.	Barley straw extractives					Rye straw extractives
		F1 <sup>a</sup>	F2 <sup>a</sup>	F3 <sup>a</sup>	F4 <sup>a</sup>	F5 <sup>a</sup>	F6 <sup>a</sup>
Steryl esters (mainly sitosteryl esters)	P35 44	5.04	23.26	24.70	11.40	23.28	16.02
All compounds identified		3.69	16.87	18.25	8.54	15.92	10.70
Steryl laurate	P37	1.45	4.02	4.25	1.87	3.26	0.95
Steryl mysistate	P39	1.08	7.02	7.24	3.38	6.90	3.56
Steryl palmitate	P41	0.46	2.64	2.70	1.38	2.30	1.86
Steryl heptadecanoate	P42	0.38	1.03	1.60	0.62	1.36	2.20
Steryl oleate	P43	0.32	2.16	2.46	1.29	2.10	2.13
Triglycerides	P45 51	1.16	8.51	10.03	5.87	8.26	5.72
All compounds identified		0.84	5.58	6.65	4.13	6.48	2.06
Tripalmitin	P46	0.18	2.01	2.64	1.46	2.75	0.23
Dipalmitoyl oleoylglycerol	P47	0.14	1.49	1.68	0.93	1.28	1.06
Triolein	P48	0.52	2.08	2.33	1.74	2.45	0.77
Total lipophilic substances		49.69	66.03	81.04	63.04	72.31	85.30
Total substances		64.75	66.50	81.79	66.12	73.79	84.65

<sup>a</sup>Fractions of the extractives obtained by extraction with toluene–ethanol (2:1, v/v; F1), chloroform (F2), MTBE (F3), hexane–acetone (2:1, v/v; F4), and dichloromethane (F5) from the barley straw and MTBE (F6) from rye straw for 12 h in a Soxhlet apparatus.

N indicates not detectable.

extracts contained 68.7–75.8% lipophilic substances, comprising mainly free acids (25.8–48.4%), waxes (9.4–27.0%), sterols (4.1–8.0%), sterol esters (2.6–5.1%), and triglycerides (3.3–11.0%). Minor amounts of diglycerides (0.3–0.5%) and resin acid (0.5–3.1%) were also quantitatively determined in the extractives.

In recent years, biological approaches have been paid more attention to reduce the influence of extractives. Wheat straw was treated with a white-rot fungus of *Phanerochaete chrysosporium* ME446 by Qin et al. [36], and the lipophilic and hydrophilic extractives from the control and biotreated samples were analyzed by GC and GC-MS. It was found that biotreatment of wheat straw could alter the chemical composition of both the lipophilic and the hydrophilic extractives. More lipophilic substances such as wax, glycerides, and steryl esters were degraded into the corresponding components, resulting in much higher concentrations of fatty acids and sterols.

### 3.2.1.2 Phenolic Compounds

Phenolic substances are widely distributed in plants such as wheat, oat, and barley. Particularly, cinnamic acids have been found in various combined forms, such as with

glycerides and polysaccharides in cell walls [37]. Because phenolic acids and aldehydes released during the Soxhlet extractions are nonvolatile compounds, they were analyzed by HPLC at 280 nm. The content of phenolic acids and aldehydes, lignan, and other compounds identified in the wheat straw extractives are given in Table 3.5 [21, 35]. Based on comparison with the authentic compounds, the phenolics compounds were estimated to comprise only 0.48–3.62% of the total extractives. No significant difference in the content of phenolic substances was detected among F1–F4 extracted with organic solvent. *p*-coumaric acid (0.042–0.050%), ferulic acid (0.022–0.041%), syringaldehyde (0.029–0.066%), vanillin (0.024–0.040%), vanillic acid (0.028–0.056%), and syringic acid (0.020–0.054%) were determined as the major compounds, whereas protocatechuic acid, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid, gallic acid, and 1-naphthoic acid appeared in minor amounts. In addition, other phenolics compounds were found to be present in trace amounts in the extractives. In contrast, the content of phenolics substances in F5–F10 extracted with hot water are relatively high, ranging between 0.91% in F5 and 3.62% in F10, although their content increased with the increase of treating temperature from 80 °C to 95 °C. The reason for this increasing trend

**TABLE 3.4** The Yield (% Dry Straw) and Chemical Composition (% Extractives) of Water-soluble Extractives in Wheat Straw [35]

Yield/composition	F1 <sup>a</sup>	F2 <sup>a</sup>	F3 <sup>a</sup>	F4 <sup>a</sup>	F5 <sup>a</sup>	F6 <sup>a</sup>
Yield	0.48	0.50	0.54	0.56	0.62	0.60
Chemical composition						
Free fatty/resin/other acids (P1 22)	50.46	45.46	37.29	28.12	33.56	27.32
Compounds analysed	44.53	39.05	29.82	24.79	29.63	25.32
Decanoic acid, C10:0 (P1)	2.56	2.20	2.12	1.85	1.43	1.31
Dodecanoic acid, C12:0 (P3)	1.31	0.88	0.72	0.36	0.66	0.21
Azelaic acid (P4)	2.06	2.08	1.13	1.20	2.36	1.54
Tetradecanoic acid, C14:0 (P5)	3.49	3.67	2.81	0.65	2.91	1.38
Pentadecanoic acid, C15:0 (P7)	4.86	4.59	3.05	2.12	3.33	2.44
Hexadecenoic acid, C16:1 (P8)	4.32	3.66	2.78	2.74	3.56	2.48
Heptadecanoic acid, C17:0 (P9)	2.88	2.50	1.12	3.08	2.98	2.99
Linoleic acid, C18:2 (P10)	0.51	0.32	0.42	0.86	0.95	1.02
Oleic acid, C18:1 (P11)	5.43	4.81	3.78	4.88	5.48	4.05
Octadecanoic acid, C18:0 (P12)	1.92	1.58	0.41	0.27	0.39	0.38
Abietic acid (P14)	3.05	2.89	1.28	1.12	0.54	0.81
Heneicosanoic acid, C21:0 (P15)	9.98	8.47	8.57	4.02	3.49	4.87
Docosanoic acid, C22:0 (P17)	0.98	0.62	0.55	0.39	0.31	0.51
Tetracosanoic acid, C24:0 (P20)	1.18	0.78	1.08	1.25	1.24	1.33
Sterols (P23 26)	4.05	4.37	6.13	7.95	7.33	7.81
Compounds analysed	4.05	4.37	6.13	7.95	7.33	7.81
Cholesterol (P23)	0.11	0.12	0.12	0.23	0.16	0.15
Ergosterol (P24)	0.31	0.36	0.46	0.77	0.74	0.78
Ergosterol + sitosterol (main compound) (P25)	3.42	3.59	4.91	5.61	5.20	6.24
Stigmastanol (P26)	0.21	0.30	0.64	1.34	1.23	0.64
Waxes (P27 32)	9.36	12.53	15.59	27.03	18.93	17.13
Compounds analysed	8.97	11.36	15.29	24.88	17.17	15.82
Palmitic acid palmityl ester (P29)	0.29	0.38	0.76	1.64	1.28	0.40
Palmitic acid oleyl ester (P30)	7.02	7.46	8.57	12.12	9.83	9.74
Oleic acid oleyl ester (P32)	1.66	4.12	5.96	11.12	6.06	5.68
Diglycerides (P33 36)	0.41	0.43	0.44	0.45	0.26	0.44
Compound analysed	0.23	0.20	0.21	0.16	0.12	0.16
Dipalmitin (P33)	0.11	0.07	0.11	0.07	0.06	0.08
1,3 Distearin (P36)	0.12	0.13	0.10	0.09	0.06	0.08
Steryl esters (mainly sitosteryl esters) (P37 45)	3.12	3.66	5.26	5.61	4.89	6.87
Compound analysed	2.64	2.67	4.07	4.25	3.78	5.07

(Continued)

**TABLE 3.4** The Yield (% Dry Straw) and Chemical Composition (% Extractives) of Water-soluble Extractives in Wheat Straw [35]—cont'd

Yield/composition	F1 <sup>a</sup>	F2 <sup>a</sup>	F3 <sup>a</sup>	F4 <sup>a</sup>	F5 <sup>a</sup>	F6 <sup>a</sup>
Steryl laurate (P37)	0.22	0.23	0.46	0.58	0.33	0.60
Continued for Table 3.1	0.48	0.50	0.54	0.56	0.62	0.60
Steryl mysistate (P39)	0.42	0.37	0.66	0.68	0.57	0.75
Steryl palmitate (P41)	0.96	0.96	1.32	1.23	1.13	1.49
Steryl heptadecanoate (P43)	0.64	0.65	0.92	0.94	0.87	1.10
Steryl oleate (P45)	0.40	0.46	0.71	0.82	0.88	1.13
Triglycerides (P46–56)	3.34	5.52	7.29	7.79	6.70	10.96
Compounds analysed	2.30	2.89	3.34	3.79	3.56	5.81
Tripalmitin (P47)	0.81	0.93	1.12	1.17	1.37	1.74
Dipalmitoyl oleoylglycerol (P48)	0.25	0.27	0.35	0.52	0.46	0.83
Dioleoyl palmitoylglycerol (P49)	0.46	0.48	0.61	0.86	0.70	1.04
Triolein (P50)	0.42	0.83	0.91	0.92	0.80	1.42
Distearoyl oleoylglycerol (P51)	0.36	0.38	0.35	0.32	0.23	0.78
Total lipophilic substances	68.68	70.89	70.87	75.75	69.31	68.99
Total substances	70.74	72.97	72.00	76.95	71.67	70.53

<sup>a</sup>Represent fractions of the extractives solubilized during the treatment of the wheat straw with water at pH 7.1 for 0.5 h at 80 °C (F1), 85 °C (F2), 90 °C (F3), 95 °C (F4), and at 90 °C for 0.5 h at pH 8.0 (F5) and pH 6.0 (F6).

was probably due to the swelling cell walls of the wheat straw in the high-temperature soaking process, in which the phenolic compounds were easily released. Occurrence of relatively higher amounts of phenolic substances in F9 (2.92%) and F10 (3.62%) showed that the phenolic compounds are more easily released at pH 8.0 and 6.0 than at pH 7.1 (in distilled water). Vanillic acid, vanillin, syringaldehyde, *p*-coumaric and ferulic acids were also verified as the major compounds.

Table 3.6 exhibits the content and composition of the phenolic components in the extractives from rice straw [18, 20]. Obviously, extraction with toluene ethanol (2:1, v/v) resulted in the dissolution of noticeable amounts of phenolic compounds (8.22%), whereas extraction with petroleum ether, dichloromethane, or hexane released only trace quantities of phenolic acids and aldehydes (0.069–0.14%); chloroform extract yielded minor amounts of lignan (2.24%), and toluene ethanol extract yielded *p*-coumaric acid (1.34%).

Table 3.7 shows the content and composition of the phenolic components in the extractives from barley and rye straw [19, 20]. For the extractives of barley, the extraction with mixtures of toluene ethanol (2:1, v/v) or with hexane acetone (2:1, v/v) released higher amounts of phenolic compounds (1.89, 2.05%) compared with extractions with

any one of the solvents such as chloroform (1.49%), MTBE (0.17%), or dichloromethane (1.09%). The extraction with MTBE yielded the least amount of phenolic compounds, indicating that MTBE was a selective solvent for extraction of lipophilic extracts from straw sample. *p*-Coumaric acid (0.008–0.22%), ferulic acid (0.004–0.11%), syringaldehyde (0.012–0.67%), vanillin (0.028–0.22%), sinapic acid (0.002–0.19%), benzoic acid (0.006–0.32%), and *m*-toluic acid (0.035–0.24%) were the major compounds in the barley straw extracts. Protocatechuic acid, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, vanillic acid, ryringic acid, 1-naphthoic acid, and lignan were quantified in relatively small amounts. In addition, trace quantities of gallic acid, acetovanillone, acetosyringone, cinnamic acid, and fumric acid were also found to be present in the lipophilic extracts. The compositions of the phenolic components in the extractives from rye straw are similar with the wheat, rice, and barley straw extracts.

### 3.2.2 Spectroscopic and Thermal Characterization

Because of the complexity of the chemical composition, it is difficult to find a single technique to analyze their

**TABLE 3.5** The Content (% Dry Extractives, w/w) of Phenolic Acids and Aldehydes, Lignan, and Other Compounds Identified in the Wheat straw Extractives by HPLC [21, 35]

Phenolic acids and aldehydes, lignan, and other compounds	F1 <sup>a</sup>	F2 <sup>a</sup>	F3 <sup>a</sup>	F4 <sup>a</sup>	F5 <sup>a</sup>	F6 <sup>a</sup>	F7 <sup>a</sup>	F8 <sup>a</sup>	F9 <sup>a</sup>	F10 <sup>a</sup>
Gallic acid	0.042	0.012	0.011	0.012						
Protocatechuic acid	0.022	0.028	0.032	0.024	0.018	0.065	0.070	0.070	0.058	0.088
<i>p</i> Hydroxybenzoic acid	0.012	0.014	0.026	0.012	0.010	0.038	0.033	0.038	0.050	0.052
<i>p</i> Hydroxybenzaldehyde	0.028	0.007	0.013	0.007	0.011	0.052	0.078	0.038	0.044	0.094
Vanillic acid	0.056	0.028	0.031	0.032	0.054	0.170	0.170	0.150	0.160	0.180
Syringic acid	0.054	0.030	0.025	0.020	0.028	0.086	0.090	0.073	0.070	0.070
Vanillin	0.040	0.024	0.027	0.024	0.072	0.160	0.160	0.150	0.180	0.250
Syringaldehyde	0.066	0.034	0.029	0.032	0.180	0.500	0.440	0.380	0.460	0.890
Acetovanillone	N	0.014	0.005	N	0.020	0.048	0.072	0.055	0.070	0.062
Acetosyringone	N	N	0.007	N	0.015	0.036	0.044	0.030	0.043	0.047
<i>p</i> Coumaric acid	0.042	0.050	0.050	0.042	0.031	0.086	0.095	0.085	0.14	0.19
Ferulic acid	0.040	0.036	0.041	0.022	0.042	0.074	0.074	0.081	0.140	0.150
Sinapic acid	0.014	0.034	0.015	0.020	0.008	0.021	0.018	0.019	0.022	0.026
Cinnamic acid	0.011	0.012	0.010	0.012	0.100	0.200	0.270	0.250	0.270	0.230
Fumaric acid	0.014	0.022	N	0.062	0.046	0.110	0.120	0.091	0.130	0.110
Benzoic acid	0.036	0.044	0.011	0.020	0.008	0.018	0.010	0.017	0.021	0.026
<i>m</i> Toluic acid	0.042	0.050	0.089	0.160	0.100	0.230	0.330	0.420	0.450	0.480
1 Naphthoic acid	0.024	0.024	0.023	0.020	0.032	0.050	0.060	0.080	0.180	0.180
Lignan	0.110	0.056	0.039	0.016	0.130	0.240	0.280	0.360	0.430	0.490
Total (%)	0.65	0.52	0.48	0.54	0.91	2.18	2.41	2.39	2.92	3.62

<sup>a</sup>Represent fractions of the extractives solubilized during the treatment of the wheat straw with toluene-ethanol (2/1, v/v) for 6 h (F1), toluene-ethanol-methanol (1/1/1, v/v/v) for 6 h (F2), methyl tert-butyl ether for 6 h (F3), chloroform-methanol (2/1, v/v) for 6 h (F4), and water at pH 7.1 for 0.5 h at 80 °C (F5), 85 °C (F6), 90 °C (F7), 95 °C (F8), and at 90 °C for 0.5 h at pH 8.0 (F9) and pH 6.0 (F10).

N indicates not detectable.

structures. It is therefore thought that the most precise way to study the extractives from straw or wood is to use a combination of several techniques, each providing partial but complementary information. Among these methods, Fourier transform-infrared (FTIR) spectroscopy has received much attention for quantitative analysis of extractives over the years [29, 38, 39]. It has a major advantage over the conventional grating instruments, having more energy, excellent reproducibility, and accuracy from the laser source. With increasing use of the microcomputer, the FTIR spectroscopy is capable of manipulating spectral information (subtraction, ratioing, derivative spectra, and deconvolution). In addition, the FTIR method is nondestructive and often permits a sample to be analyzed in situ. The region between 780 and 2526 cm<sup>-1</sup> has been shown to have potential

applications in the pulp and paper industry. It has been used to determine wood resin directly in ground wood pulp [1]. Carbon-13 (<sup>13</sup>C) nuclear magnetic resonance (NMR) spectroscopy is another useful tool for studying various problems related to lipid technology. Complementary information about lipid class composition and total acyl profile can be inferred simultaneously from the same <sup>13</sup>C-NMR spectrum [31, 32]. This technique enables quantization of the extractives in terms of total fatty acids, resin acids, triglycerides, and fatty acid esters. The method was found to be as accurate as conventional methods for analyzing extractives. The advantages of the method are that it is nondestructive and analysis is fast [30]. Furthermore, thermal analysis has proved useful for analyzing lipids. Thermogravimetric analysis (TGA) can be used to monitor the weight



**TABLE 3.6** The Content (% Dry Extractives, w/w) of Phenolic Acids and Aldehydes, Lignan, and Other Compounds Identified in the Rice Straw Extractives by HPLC [18]

Phenolic acids and aldehydes, lignan, and other compounds	F1 <sup>a</sup>	F2 <sup>a</sup>	F3 <sup>a</sup>	F4 <sup>a</sup>	F5 <sup>a</sup>	F6 <sup>a</sup>
Gallic acid	0.10	0.010	N	N	N	N
Protocatechuic acid	0.23	0.034	N	N	N	0.011
<i>p</i> Hydroxybenzoic acid	0.31	0.042	N	0.013	0.011	0.008
<i>p</i> Hydroxybenzaldehyde	0.41	0.062	0.002	0.006	0.003	0.006
Vanillic acid	0.31	0.081	0.002	0.044	0.005	0.007
Syringic acid	0.17	0.038	0.003	0.005	0.003	0.003
Vanillin	0.26	0.054	0.006	0.004	0.011	0.006
Syringaldehyde	0.19	0.068	0.008	0.001	0.013	0.004
Acetovanillone	0.13	0.005	N	N	N	0.002
Acetosyringone	0.005	0.002	N	N	N	0.001
<i>p</i> Coumaric acid	1.34	0.12	0.006	0.006	0.005	0.011
Ferulic acid	0.38	0.041	0.004	0.007	0.005	0.006
Sinapic acid	0.12	0.038	N	N	N	0.002
Cinnamic acid	0.18	0.021	N	0.007	0.003	0.001
Fumaric acid	0.34	0.013	0.003	0.005	0.003	N
Benzoic acid	0.32	0.047	N	N	N	0.003
<i>m</i> Toluic acid	0.56	0.11	0.011	0.010	0.013	0.001
1 Naphthoic acid	0.62	0.021	0.011	0.010	N	0.008
Lignan	2.24	0.13	0.013	0.010	0.011	0.015
Total (%)	8.22	0.94	0.069	0.14	0.086	0.10

<sup>a</sup>Corresponding to the extractive fractions in Table 3.2.

N indicates not detectable.

loss of the extractives as they are heated, cooled, or held isothermally; and differential scanning calorimetry (DSC) was performed to determine the melting temperature and enthalpies of the extractives [40].

### 3.2.2.1 FTIR and <sup>1</sup>H and <sup>13</sup>C-NMR Spectra

Fig. 3.5 shows the FTIR spectra of the toluene ethanol extract (2:1, v/v, spectrum 1), chloroform methanol extract (2:1, v/v, spectrum 2), and MTBE extract (spectrum 3) from wheat straw by Sun and Tomkinson [41]. The absorption band at 3436 cm<sup>-1</sup> was given by the OH stretching vibration mode of sterols or monoglycerides/diglycerides or water in samples [38]. Extremely strong methylene and methyl stretching frequencies, particularly in spectra 2 and 3, appeared at 2926 and 2866 cm<sup>-1</sup>, respectively. A carboxylic carbonyl stretching peak was produced at 1712 cm<sup>-1</sup> as a

“shoulder” to the ester carbonyl stretching peak seen at 1746 cm<sup>-1</sup>. These bands arise from the carboxylic carbonyl of the free fatty acids or the resin acids that absorb at 1712 cm<sup>-1</sup> and the ester carbonyl of triglycerides or steryl esters or waxes that frequently absorb at 1746 cm<sup>-1</sup>. The C=C *cis* stretching, arising from double bonds in unsaturated fatty acids such as linoleic acid and oleic acid and their esters or in sterols, produced an absorption band of 1646 cm<sup>-1</sup>. Two bands at 1474 and 1388 cm<sup>-1</sup> were due to the CH absorption bending vibration of CH<sub>2</sub> and CH<sub>3</sub>, respectively. A broad, strong carbon single-bonded oxygen (C O) stretching vibration appears at 1268 cm<sup>-1</sup>. This band could be an interaction band between carbon single-bonded oxygen stretching and in-plane carbon single-bonded hydroxyl bending in carboxylic acids. The broad band appearing at 1182 cm<sup>-1</sup> may be arising from saturated esters in conformity with ester carbonyl stretching. The weak shoulder

**TABLE 3.7** The Content (% Dry Extractives, w/w) of Phenolic Acids and Aldehydes, Lignan, and Other Compounds Identified in the Barley and Rye Straw Extractives by HPLC [19, 20]

Phenolic acids and aldehydes, lignan, and other compounds	F1 <sup>a</sup>	F2 <sup>a</sup>	F3 <sup>a</sup>	F4 <sup>a</sup>	F5 <sup>a</sup>	F6 <sup>a</sup>
Gallic acid	0.050	N	N	0.055	0.025	N
Protocatechuic acid	0.078	0.008	0.011	0.12	0.045	0.022
<i>p</i> Hydroxybenzoic acid	0.065	0.012	0.010	0.086	0.047	0.018
<i>p</i> Hydroxybenzaldehyde	0.059	0.004	0.006	0.041	0.037	0.011
Vanillic acid	0.098	0.009	0.023	0.067	0.043	0.023
Syringic acid	0.034	0.036	0.003	0.043	0.032	0.010
Vanillin	0.078	0.12	0.028	0.11	0.084	0.020
Syringaldehyde	0.22	0.67	0.012	0.16	0.11	0.017
Acetovanillone	0.043	0.003	N	0.005	0.005	0.008
Acetosyringone	0.065	0.005	N	0.008	0.006	0.005
<i>p</i> Coumaric acid	0.22	0.026	0.008	0.20	0.12	0.043
Ferulic acid	0.11	0.013	0.004	0.066	0.031	0.018
Sinapic acid	0.19	0.003	0.002	0.074	0.051	0.016
Cinnamic acid	0.030	0.024	N	0.050	0.022	0.003
Fumaric acid	0.033	0.006	N	0.32	0.078	0.001
Benzoic acid	0.19	0.028	0.006	0.32	0.19	0.021
<i>m</i> Toluic acid	0.14	0.21	0.035	0.24	0.11	0.003
1 Naphthoic acid	0.094	0.042	0.018	0.065	0.023	0.33
Lignan	0.090	0.27	0.008	0.020	0.034	0.040
Total (%)	1.89	1.49	0.17	2.05	1.09	0.57

<sup>a</sup>Corresponding to the extractive fractions in Table 3.3.

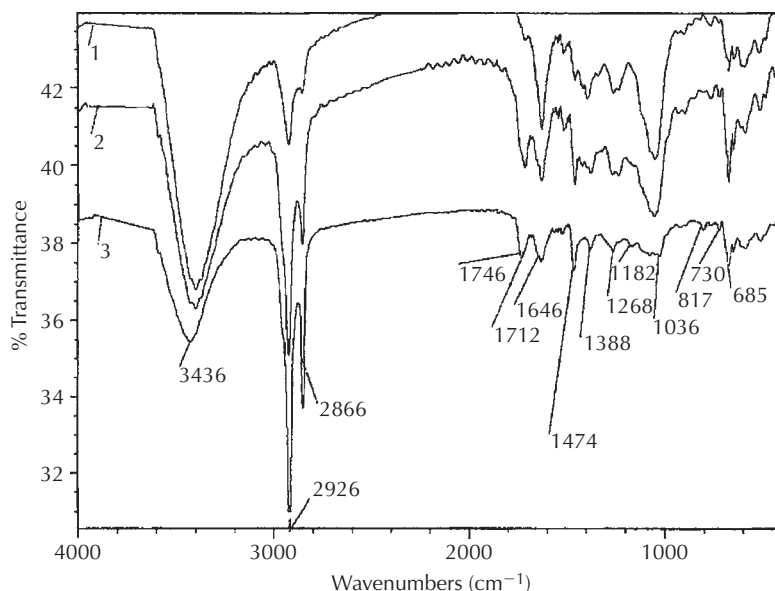
N indicates not detectable.

appearing at  $1122\text{ cm}^{-1}$  in spectrum 3 may arise from aliphatic ether stretching [29]. The strong band at  $1045\text{ cm}^{-1}$  in spectra 1 and 2 are attributed to the symmetrical stretching of an ether bond (C-O-C), implying that the extractions with toluene-ethanol or chloroform-methanol may dissolve some of the low-molecular-weight polysaccharides. The far peaks at low frequencies of  $817$  and  $730\text{ cm}^{-1}$  are assigned to the ring out-of-plane carbon single-bonded hydrogen bending vibrations associated with two adjacent hydrogen atoms and in-plane methylene ( $\text{CH}_2$ ) rocking absorption in straight-chain lipids, respectively [38].

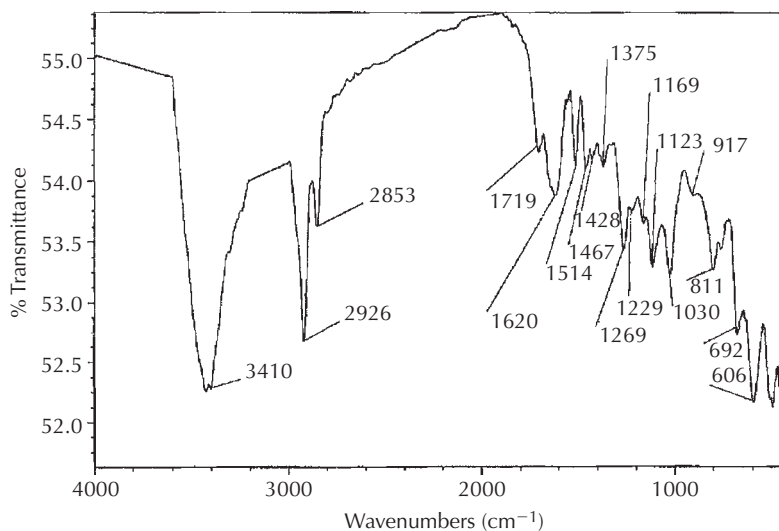
Treatment of the straw with water [35, 41] is expected to release materials from within the wall structure, including, among others, condensed tannins and phenolics compounds. The spectrum produced by this extract is presented in Fig. 3.6. In addition to the main vibration bands for lipophilic substances, the spectrum shows the typical ring

skeletal bands at about  $1620$ ,  $1514$ , and  $1428\text{ cm}^{-1}$  for phenolics compounds. Thus, it may be recognized that the water-soluble components of wheat straw comprise noticeable amounts of aromatic substances, as shown by the predominance of ring vibrations in the spectrum. This observation was also confirmed by the results obtained by HPLC analyses. The FTIR spectra of rice and barley straw extractives have the similar absorption bands with wheat straw [19, 22, 42, 43].

The chemical components of extractives can also be identified using Raman spectroscopy [44]. The resolution of Raman microscopy is adequate to study distribution of substances in cellulosic fibers of about  $20\text{ }\mu\text{m}$  in width. This way the distribution of extractives in different fiber wall layers has been analyzed from fiber cross-sections [45]. On the other hand, atomic force microscopy (AFM) has been used to characterize surface properties of wood, pulp, and



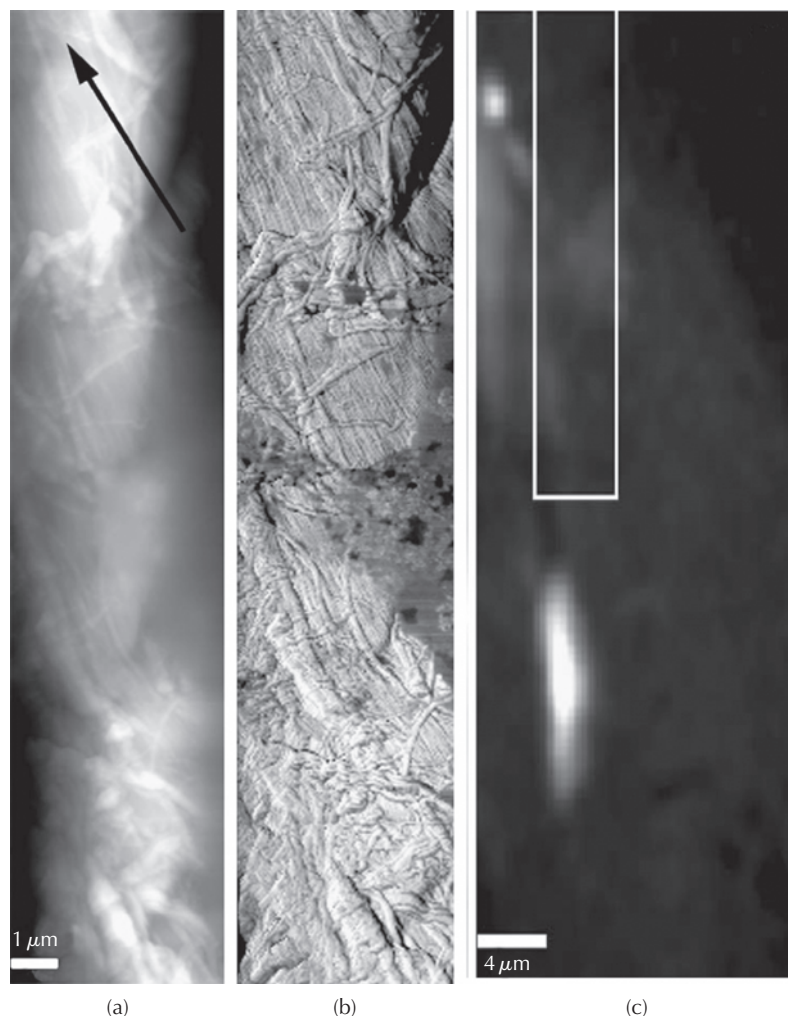
**FIGURE 3.5** Fourier transform infrared spectra of extractives obtained by extraction with toluene ethanol (2:1, v/v, spectrum 1), chloroform methanol (2:1, v/v, spectrum 2), and methyl tert butyl ether (spectrum 3) from wheat straw for 6 h in a Soxhlet apparatus [41].



**FIGURE 3.6** Fourier transform infrared spectrum of extractives solubilized during the treatment of wheat straw with water at 98 °C for 4 h and isolated by extraction with methyl tert butyl ether at room temperature [41].

paper [46]. Therefore, combining confocal Raman microscopy (CRM) with AFM in the same instrument enables the link between the high-resolution topographical information acquired with AFM and the chemical information obtained by CRM. Österberg et al. [47] showed that combining CRM and AFM could be applied to study wood extractives on cellulose surfaces. Wood resin was extracted from unbleached thermo mechanical pulp (TMP) pulp using hexane, dissolved in acetone, precipitated in water, and dialyzed for 3 days to remove the traces of acetone. The concentration of the final dispersion was 0.24 g/l. The chemical composition of the resin was 48% triglycerides,

23% sterol esters, 13% fatty acids, 13% resin acids, 2% sitosterols, and 1% lignans. A total of 120  $\mu$ l of the dispersion was pipetted on a cellulose paper sample. Fig. 3.7 shows AFM height (a) and phase contrast (b) images of the precipitated extractives on the paper sample, and Raman spectral images (c) of extractives obtained from basis analysis of the two-dimensional spectra array. One cellulose fiber is partly visible in the AFM image. As expected, the AFM height image gives but little information concerning precipitated material on the surface due to the roughness of the fiber. Areas with a different phase contrast are seen in the center of the image, which may be assigned to precipitated



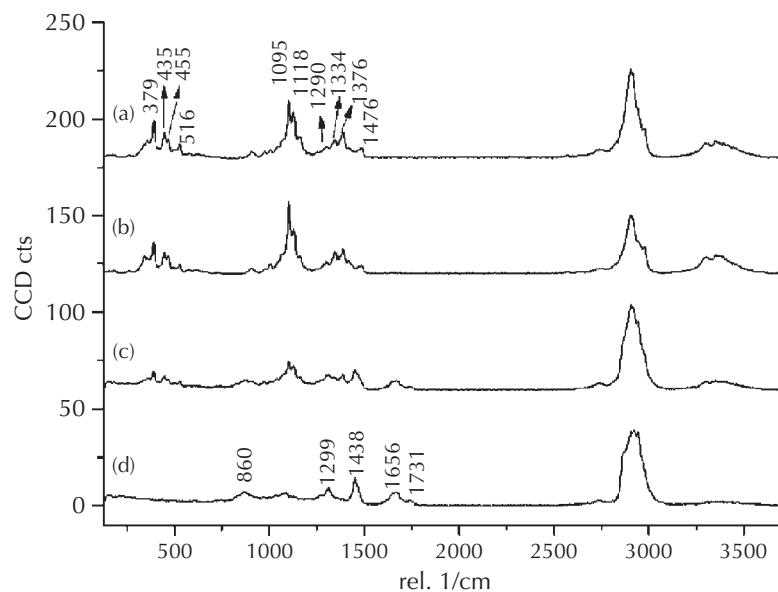
**FIGURE 3.7** Atomic force microscopy height (a) and phase contrast (b) images of cellulose with precipitated extractives. The image size was  $5\ \mu\text{m} \times 20\ \mu\text{m}$ . Raman spectral images obtained from basis analysis of the two dimensional spectra array, (c) distribution of extractives. Image size  $20\ \mu\text{m} \times 40\ \mu\text{m}$  [47].

extractives. These areas appear darker in the phase image than the cellulose microfibrils. To discriminate the chemical composition of the sample, an enlarged area of the same surface area was imaged in Raman spectral imaging mode. As shown in Fig. 3.8., the spectrum c shows additional bands arising from hexane-extract of wood [48], the low intensity of the bands from the extractives when compared with the intensity of the bands from cellulose illustrates that the extractives formed only a thin layer on the fiber surface. By subtracting the cellulose-originating bands from the spectrum c, a cellulose-free spectrum of extractives could be obtained (spectrum d). The bands in this spectrum could be assigned to originate mainly from triglycerides and fatty acids (Table 3.8), which were the main components of the wood extract. The wood extractives are unevenly distributed in the cellulose fibers, as can be seen from the spectral image of extractives in Fig. 3.7c. Two extractive-rich regions were observed, one close to the center and another in the upper left corner of the spectral image. Comparing

the intensity of the spectral images (Fig. 3.7c) to the AFM phase image (Fig. 3.7b), a clear correlation can be observed; the darker areas in the phase image can be assigned to extractives consisting of triglycerides, fatty acids, steryl esters, and resin acids, whereas the fibrillar structures originate from cellulose, which is mainly oriented in parallel to the fiber axis direction. The greatest advantage of coupling AFM and Raman is that the same area on the sample is measured. In addition, it is a fast, easy, and reliable way to acquire both morphological and chemical information from the sample [47].

The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra of the hexane extract from wheat straw are given by Sun and Tomkinson [41]. The  $^1\text{H}$ -NMR spectrum of hexane extract from wheat straw is shown in Fig. 3.9. The most intense signal, occurring at 1.28 ppm, is attributed to the methylene aliphatic protons; the signal centered at 0.87 ppm is assigned to methyl protons. Minor signals from additional structural features [protons on carbons adjacent to carbonyl in esters





**FIGURE 3.8** Characteristic Raman spectra recorded on the paper sample with precipitated wood extractives. (a) and (b) show characteristic Raman spectra of cellulose with different orientations of fibrils, (c) superposition of Raman bands origin from cellulose and hexane extract of wood, (d) cellulose free Raman spectrum of extractives [47].

**TABLE 3.8** Assignment of the Raman Bands of Cellulose and Extractives [47]

Raman shift (cm <sup>-1</sup> )	Vibrational mode	Origin
3500 3200	OH stretch	Cellulose
3000 2800	CH, CH <sub>2</sub> stretch	Cellulose Triglycerides Steryl esters Fatty acids Resin acids Sterols
1731	C O stretch	Triglycerides Steryl esters
1656	C C stretch	Unsaturated fatty acids Resin acids Sterols
1476	HCH and HOC bend	Cellulose
1438	CH <sub>2</sub> , CH <sub>3</sub> deformation	Triglycerides Steryl esters Fatty acids
1376, 1334	HCC, HCO and HOC bend	Cellulose
1299	CH <sub>2</sub> deformation	Triglycerides Fatty acids
1290	HCC and HCO bend	Cellulose
1118, 1095	CC and CO stretch	Cellulose
860	CC stretch	Triglycerides Fatty acids
516 379	Skeletal (CCC, COC, OCC and OCO) bend	Cellulose

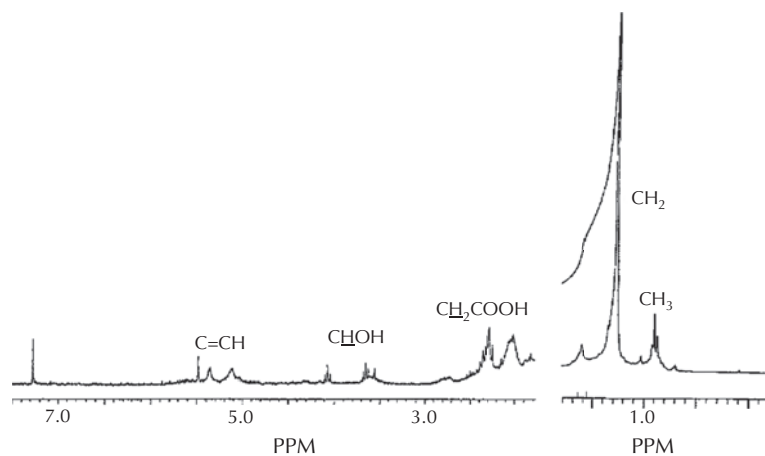


FIGURE 3.9  $^1\text{H}$  nuclear magnetic resonance spectrum of hexane extract from wheat straw [41].

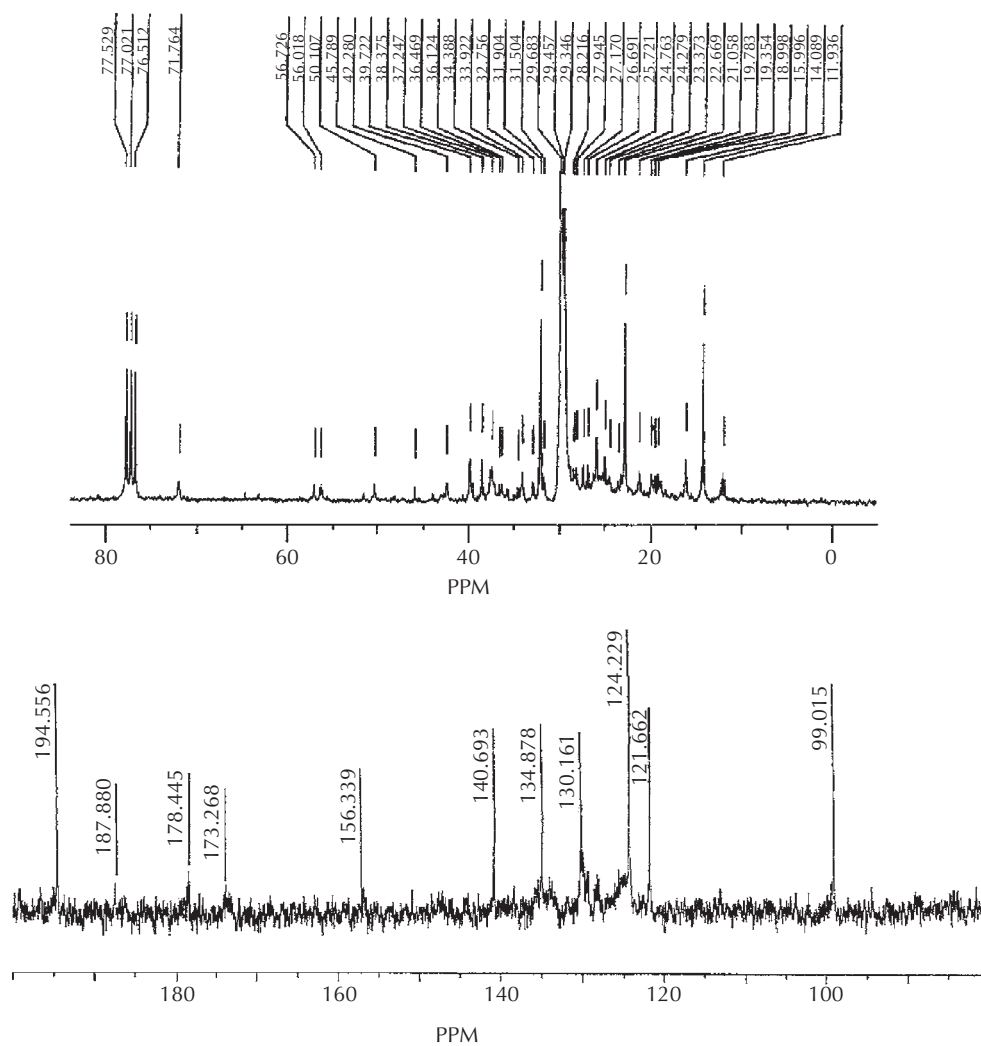
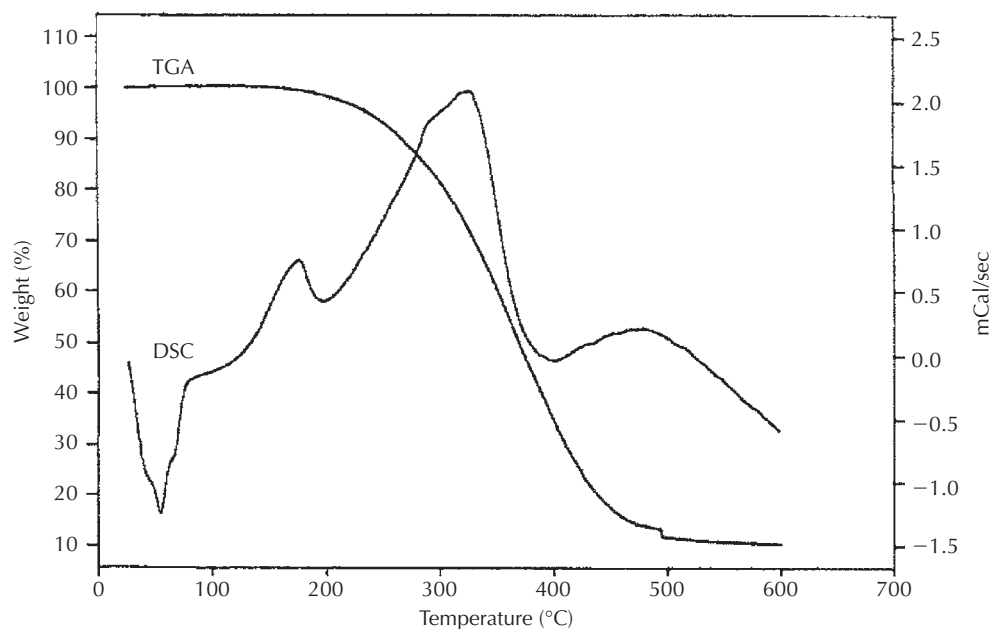


FIGURE 3.10  $^{13}\text{C}$  nuclear magnetic resonance spectrum of methyl *tert* butyl ether extract from wheat straw [41].



**FIGURE 3.11** Thermogram of hexane extract TGA, thermogravimetric analysis; DSC, differential scanning calorimetry [41].

( $\text{CH}_2\text{-C=O}$ ) or carboxyl in fatty acids ( $\text{CH}_2\text{-COOH}$ ), hydroxyl groups ( $\text{CHOH}$ ), and alkene ( $\text{CH=C}$ ) protons] appear at 2.0–2.4, 3.6–4.1, and 5.1–5.5 ppm, respectively [10]. The peak at 7.26 ppm is residual chloroform present in  $\text{CDCl}_3$  and should be ignored.

Fig. 3.10 shows the  $^{13}\text{C}$ -NMR spectrum of MTBE extract from wheat straw. The carbonyl group is obviously characterized by a strong signal at 194.6 ppm. Signals at 187.9, 178.4, and 173.3 ppm correspond to carbonyl groups of resin acids, fatty acids, and fatty acid/steryl esters, respectively. The signals at 156.3, 140.7, 134.9, 124.2, and 121.7 ppm originate from  $>\text{C=}$  in sterols and steryl esters. The signal attributed to an unsaturated carbon double bond ( $-\text{CH=CH}-$ ) in fatty acids or fatty acid esters appears at 130.2 ppm. The groups of  $\text{CH}_2\text{-O}$  in glycerol carbons of triglycerides and  $>\text{CH-O}$  in sterol or steryl esters were identified with signals between 75 and 55 ppm. A small signal at 64.3 ppm (alkoxy C1 in ester) and a reduced signal at 63.0 ppm (alkoxy C1 in alcohol) were observed in the spectrum [31]. The strong signals assigned to methyl and methylene groups appear in the spectrum between 14.1 and 39.7 ppm. The peak at 14.1 ppm is the methyl end of the chain. The peaks at 22.7, 31.9, and 29.3 ppm were methylene units successively farther from the methyl group. The large peak at 29.7 ppm is long internal methylene. The peak at 19.8 ppm is indicative of methyl branches on a straight methylene backbone, and the peak at 32.8 ppm is the methane associated with that methyl branch [49].

### 3.2.2.2 Thermal Analysis

The thermal properties of the extractives were studied by thermogravimetric analysis and differential scanning

calorimetry [41]. The thermogram of hexane extract from wheat straw is shown in Fig. 3.11. As can be seen, the extract started to decompose at about 200 °C, and its maximum rate of weight loss occurred between 300 °C and 450 °C. The decomposition temperature at 10% weight loss appeared at 271 °C. When the temperature increased to 300 °C, the weight loss accounted for 15.6%, and at 450 °C, it increased significantly to 83.0%. There was little nonvolatile residue at 600 °C (<10% of the total extract material). In Fig. 3.11, DSC was also performed to determine the melting temperatures and enthalpies of the extract. The melting temperature, due to a solid liquid phase transition from the hexagonal crystal structure to a melt, occurred at about 54 °C. This is consistent with previously reported values for wood resins from recycled fiber [40]. In addition, the DSC thermogram of the extractives produced one big, broad exothermic peak with a maximum at 328 °C.

## REFERENCES

- [1] B.B. Sithole, Modern methods for the analysis of extractives from wood and pulp: a review, *Appita* 45 (1992) 260–264.
- [2] T. Chen, C. Breuil, S. Carriere, J.V. Hatton, Solid phase extraction can rapidly separate lipid classes from acetone extracts of wood and pulp, *Tappi J.* 77 (1994) 235–240.
- [3] R. Ekman, B. Holmbom, Analysis by gas chromatography of the wood extractives in pulp and water samples from mechanical pulping of spruce, *Nordic Pulp Pap. Res. J.* 1 (1989) 16–24.
- [4] A. Gutiérrez, J.C. Del Ril, F.J. Gonzalez Vila, F. Martin, Chemical composition of lipophilic extractives from *Ecalyptus globules* labill. *Wood, Holzforschung* 53 (1999) 481–486.

- [5] A. Gutiérrez, J.C. Del Río, F.J. Gonzalez Vila, F. Martín, Analysis of lipophilic extractives from wood and pith deposits by solid phase extraction and gas chromatograph, *J. Chromatogr. A* 823 (1998) 449–455.
- [6] J.E. Atchison, Twenty five years of global progress in non wood plant fiber repulping, *Tappi J.* 79 (1996) 87–95.
- [7] B.E. Juniper, The structure and chemistry of straw, *Agric. Progress* 54 (1979) 18–27.
- [8] H.J. Chaves das Neves, E.M.M. Gaspar, Identification of active compounds in wheat straw extractives with allelopathic activity by HRGC MS and HRGC FTIR, *J. High Res. Chromatogr.* 13 (1990) 550–554.
- [9] B. Lindgren, T. Norin, The chemistry of resin, *Sven. Papperstidn.* 72 (1969) 143–153.
- [10] A.F.A. Wallis, R.H. Weame, Characterization of resin in radiata pine woods, bisulfite pulps and mill pith samples, *Appita* 50 (1997) 409–414.
- [11] N. Levitin, The extractives of birch, aspen, elm, and maple: review and discussion, *Pulp Paper Mag. Can.* 71 (1970) 81–86.
- [12] N. Dunlop Jones, H. Jialing, L.H. Allen, An analysis of the acetone extractives of the wood and bark from fresh trembling aspen: implications for deresination and pitch control, *J. Pulp Pap. Sci.* 17 (1991) 60–66.
- [13] D.W. Reeve, A.B. McKague, Chlorinated organic matter in bleached chemical pulp production. Part VII Characterization of extractives, *J. Pulp Pap. Sci.* 17 (1991) 115–118.
- [14] A. Demirbas, Analysis of beech wood fatty acids by supercritical acetone extraction, *Wood Sci. Technol.* 25 (1991) 365–370.
- [15] S.B. Hawthorn, Analytical scale supercritical fluid extraction, *Anal. Chem.* 62 (1990) 633A–642A.
- [16] H.B. Lee, T.E. Peart, J.M. Carron, GC and MS determination of some resin and fatty acids in pulpmill effluents as their pentafluorobenzyl ester derivatives, *J. Chromatogr. A* 498 (1990) 367–380.
- [17] R.C. Sun, J. Tomkinson, Comparative study of organic solvent and water soluble lipophilic extractives from wheat straw I: yield and chemical composition, *J. Wood Sci.* 49 (2003) 47–52.
- [18] B. Xiao, X.F. Sun, R.C. Sun, Extraction and characterization of lipophilic extractives from rice straw I: chemical composition, *J. Wood Chem. Technol.* 21 (2001) 397–411.
- [19] R.C. Sun, X.F. Sun, Separation and characterization of lipophilic extracts from barley straw, *Sep. Sci. Technol.* 36 (2001) 3027–3048.
- [20] X.F. Sun, R.C. Sun, Comparative study of methyl *tert* butyl ether extractives from rye and rice straw, *Wood Fiber Sci.* 33 (2001) 386–394.
- [21] R.C. Sun, X.F. Sun, Identification and quantization of lipophilic extractives from wheat straw, *Ind. Crop. Prod.* 14 (2001) 51–64.
- [22] R.C. Sun, X.F. Sun, J. Tomkinson, A convenient one step method for analysis of lipophilic extractives from agricultural residues, *Recent Res. Dev. Agric. Food Chem.* 5 (2001) 25–42.
- [23] Anonymous, Waters sep pak cartridge applications bibliography, fourth ed., Milford, USA, 1986.
- [24] I.D. Suckling, S.S. Gallagher, R.M. Ede, A new method for softwood extractives analysis using high performance liquid chromatography, *Holzforschung* 44 (1990) 339–345.
- [25] D.E. Richardson, J.B. Bremner, O. Grady, Quantitative analysis of total resin acids by high performance liquid chromatography of their coumarin ester derivatives, *J. Chromatogr.* 595 (1992) 155–159.
- [26] J. Sjöström, B. Holmbom, Size exclusion chromatography of deposits in pulp and paper mills, *J. Chromatogr.* 411 (1987) 363–367.
- [27] M. Sandström, M.A. Norborg, A. Ericsson, Application of thin layer chromatography to process control in the pulp and paper field, *J. Chromatogr. A* 730 (1996) 373–377.
- [28] T.L. Chang, Analysis of tall oil by gel permeation chromatography, *Anal. Chem.* 40 (1968) 989–992.
- [29] E.M.A. Ajuong, M.C. Breese, Fourier transform infrared characterization of *Pai* wood (*Azela africana* smith) extractives, *Holz Roh. Werkst.* 56 (1998) 139–142.
- [30] D. Suckling, R.M. Ede, A quantitative  $^{13}\text{C}$  Nuclear Magnetic Resonance method for the analysis of wood extractives and pitch sample, *Appita* 43 (1990) 77–80.
- [31] F.D. Gunstone, High resolution  $^{13}\text{C}$  NMR spectra of long chain acids, methyl esters, glycerol esters, nitriles, amides, alcohols and acetates, *Chem. Phys. Lipids* 66 (1993) 189–193.
- [32] I. Medina, R. Sacchi, S. Aubourg,  $^{13}\text{C}$  Nuclear Magnetic Resonance monitoring of free fatty acid release after thermal processing, *JAOCS* 71 (1994) 479–482.
- [33] G. Marques, A. Gutierrez, J.C. Del Río, Chemical composition of lignin and lipids from tagasaste (*Chamaecytisus proliferus* spp. palmensis), *Ind. Crop. Prod.* 28 (2008) 81–87.
- [34] H.J. Chaves Das Neves, E.M.M. Gaspar, HRGC MS and HPLC MS identification of new ketosterols in an extract of wheat straw, *J. High Res. Chromatogr.* 18 (1995) 299–303.
- [35] R.C. Sun, D. Salisbury, J. Tomkinson, Chemical composition of lipophilic extractives released during the hot water treatment of wheat straw, *Bioresource Technol.* 88 (2003) 95–101.
- [36] M.H. Qin, Q.H. Xu, Z.Y. Shao, Y.J. Fu, X.M. Lu, P.J. Gao, B. Holmbom, Effect of bio treatment on the lipophilic and hydrophilic extractives of wheat straw, *Bioresource Technol.* 100 (2009) 3082–3087.
- [37] A.C. Salomonsson, O. Theander, P. Åman, Quantitative determination by GLC of phenolic acids as ethyl derivatives in cereal straws, *J. Agric. Food Chem.* 26 (1978) 830–835.
- [38] Y.B.C. Man, G. Setiowaty, Application of Fourier transform infrared spectroscopy to determine free fatty acid contents in palm olein, *Food Chem.* 66 (1999) 109–114.
- [39] Y.B.C. Man, M.H. Moh, F.R. Van de Voort, Determination of free fatty acids in crude palm oil and refined bleached deodorized palm olein using Fourier transform infrared spectroscopy, *JAOCS* 76 (1999) 485–490.
- [40] X.Y. Guo, M. Douek, Analysis of deposits/stickies from newsprint mills using recycled fibre, *J. Pulp Pap. Sci.* 22 (1996) 431–438.
- [41] R.C. Sun, J. Tomkinson, Comparative study of organic solvent soluble and water soluble lipophilic extractives from wheat straw 2: spectroscopic and thermal analysis, *J. Wood Sci.* 48 (2002) 222–226.
- [42] R.C. Sun, X.F. Sun, B. Xiao, Extraction and characterization of lipophilic extractives from rice straw II. Spectroscopic and thermal analysis, *J. Wood Chem. Technol.* 22 (2002) 1–9.
- [43] X.F. Sun, R.C. Sun, Spectroscopic characterization of extractives isolated with MTBE from straws, *Tappi J.* 2 (2003) 23–26.
- [44] A. Holmgren, B. Bergström, R. Gref, A. Ericsson, Detection of pinosylvin in solid wood of scots pine using Fourier Transform Raman and Infrared Spectroscopy, *J. Wood Chem. Technol.* 19 (1999) 139–150.
- [45] U.P. Agarwal, Proceedings of the Thirteenth International Symposium on Wood Fibre and Pulping Chemistry, Victoria, Australia, *Appita* 2 (2005) 377–384.
- [46] H. Niemi, H. Paulapuro, R. Mahlberg, Review: application of scanning probe microscopy to wood, fibre and paper research, *Pap. Puu Pap. Tim.* 84 (2002) 389–406.



- [47] M. Österberg, U. Schmidt, A.S. Jääskeläinen, Combining confocal Raman spectroscopy and atomic force microscopy to study wood extractives on cellulose surfaces, *Colloids Surf. A* 291 (2006) 197–201.
- [48] M. Nupponen, S. Willfor, A.S. Jaaskelainen, A. Sundberg, T. Vuorinen, A UV resonance Raman (UVR) spectroscopic study on the extractable compounds of Scots pine (*Pinus sylvestris*) wood Part I: lipophilic compounds, *Spectrochim. Acta A* 60 (2004) 2953–2961.
- [49] B.J. Musser, P.K. Kilpatrick, Molecular characterization of wax isolated from a variety of crude oils, *Energy Fuels* 12 (1998) 715–725.

# Hemicelluloses

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## 4.1 INTRODUCTION

The world is facing tremendous challenges: the future shortage of natural energy resources, world-wide environmental problems that require replacement of petroleum-based products, and the demand for healthy food and medicines. Developing clean, safe bio-based materials and energy from the most plentiful, renewable biomass resources to replace the traditional material and energy sources is the solution to overcome these challenges. This has led to immense interest in the research of polysaccharide, including hemicellulose. During the last decade, considerable efforts by scientists from universities and research institutes as well as industrial companies have been devoted to research into hemicelluloses from renewable sources. The potential for utilizing hemicelluloses is vast, which has been emphasized many times by leading polysaccharide scientists. However, hemicelluloses have not yet been applied on an industrial scale.

Hemicelluloses are the second most abundant biopolymer in the plant kingdom after cellulose. They differ from cellulose in the main cell wall constituent, which is a highly uniform 1→4-β-linked polyglucan. Hemicelluloses represent a type of hetero-polysaccharides with complex structures containing glucose, xylose, mannose, galactose, arabinose, fucose, glucuronic acid, and galacturonic acid in various amounts depending on the source. The increasing knowledge and the growing willingness to develop new biopolymer-based materials will lead to an increasing application of hemicelluloses and their derivatives from sustainable lignocellulosic biomass such as trees, grasses, cereals, herbs, cereal straws, and other plants [1]. Earlier research activities in the field of hemicelluloses were focused on utilizing plant biomass by conversion into sugar, chemicals, and fuel as sources of heat energy. Now, because of their structural varieties and diversities, they are also attractive as biopolymers, which can be utilized in their native and modified forms in various areas, including food and nonfood applications.

Although known for a long time, hemicelluloses from agricultural straws did not attract much attention until the last decade. Straw is an agricultural byproduct that has not been used as industrial raw material on a significant scale in the world. Depending on the plant species and the cell wall composition, according to the various functions in plant cells, tissues, and organs, the hemicellulosic components might differ greatly in content and structural features [2], such as 32%, wheat straw; 32%, barley straw; 27%, oat straw; 31%, rye straw; 25%, rice straw; 23%, sunflower husk; 22%, sugarcane rind; and 37%, corn cobs (CCs) [3]. Therefore, the large amounts of straw hemicelluloses are significant enough to be considered as a complementary source of raw material for different industries such as papermaking, baking, and food as well as other nonfood industries.

This review article updates and extends previous reviews [4, 5] and will focus in particular on newly investigated straw hemicelluloses, especially regarding their location in straw, occurrence, isolation methods, structural features, physicochemical and various functional properties of hemicelluloses as well as the interactions with cellulose. Attention will also be paid to the modification of isolated hemicelluloses or hemicellulosic materials and the application possibilities of hemicelluloses and their derivatives, including their use for the production of composite materials and other biomaterials.

## 4.2 OCCURRENCE, NATURE, AND CLASSIFICATION

Hemicelluloses, one of the most abundant natural polysaccharides, comprise roughly one-fourth to one-third of most plant materials. Together with cellulose and pectic polysaccharides, hemicelluloses belong to the building components of the cell walls of higher plants, where they are associated with varying levels of proteins and phenolics [6]. They are known to occur in softwood, hardwood, and annual plants such as grass, herbs, and cereal including cereal

grains and cereal straw. Hemicelluloses are generally defined as being polysaccharides that can be extracted by water or aqueous alkali from plant tissue [7, 8]. However, hemicelluloses are the most complex components in the cell wall of woods, cereal straws, and grasses. They form hydrogen bonds with cellulose, covalent bonds (mainly  $\alpha$ -benzyl ether linkages) with lignin, and ester linkages with acetyl units and hydroxycinnamic acids. They are branched polymers of low molecular weight ( $M_w$ ) with a degree of polymerization (DP) of 80–200. Their general formulas are  $(C_5H_8O_4)_n$  and  $(C_6H_{10}O_5)_n$ , which are called pentosans and hexosans, respectively [9]. They can be comprised of a wide variety of monosaccharides including xylose, arabinose, glucose, galactose, mannose, fucose, glucuronic acid, and galacturonic acid depending on the source. The most common hemicelluloses, largely found in hardwood or annual plants, are comprised of a 1,4- $\beta$ -D-xylopyranosyl main chain with a varying number of side chains based on L-arabinofuranosyl, 4-O-methyl-D-glucuronopyranosyl, L-galactopyranosyl, or D-glucuronopyranosyl units. Hemicelluloses isolated from hardwood and annual plants differ from one another [10]. The main hemicelluloses found in hardwood are partially acetylated (4-O-methyl-D-glucuronopyranosyl)-D-xylans and these are often simply called *xylans*. The hemicelluloses found in annual plants such as maize, rice, oats, sunflower, rye, barley, and wheat straws, are generally more structurally diverse and complex. These plant hemicelluloses have a 1,4- $\beta$ -D-xylopyranosyl main chain that can be heavily branched with Xylp- (xylopyranose), Araf- (arabinofuranose), Galp- (galactopyranose) mono-, di-, and trisaccharide side chains. These plant hemicelluloses can be neutral or acidic depending on whether they contain 4-O-methyl-D-glucuronopyranosyl or D-glucuronopyranosyl substituents. Generally, the two predominant monosaccharides in these annual plant hemicelluloses are xylose and arabinose, and they are thus termed as *arabinoxylans*.

### 4.2.1 Occurrence, Nature, and Classification of Hemicelluloses

Based on the current stage of knowledge, hemicelluloses are usually divided into four general groups of structurally

different polysaccharide type: (1) xyloglycans (xylans); (2) mannoglycans (mannans); (3) xyloglucans (XG); and (4) mixed-linkage  $\beta$ -glucans [11]. Xylan-type polysaccharides are the main hemicellulose components of secondary cell walls constituting about 20–30% of the biomass of dicotyl plants (hardwoods and herbaceous plants). In some tissues of monocotyl plants (grasses and cereals), xylans occur up to 50% [1]. In most cases, xylans consist of a  $\beta(1\rightarrow4)$ -D-xylopyranose backbone with carbohydrate chains on the 2- or 3-position, which comprise D-glucuronic acid or its 4-O-methyl ether, L-arabinose and/or various oligosaccharides, composed of D-xylose, L-arabinose, D- or L-galactose and D-glucose (Fig. 4.1). Based on the hitherto-reported review articles on the primary structure of xylans from various plant tissues, xylan-type polysaccharides can be divided into homoxylans (X) and heteroxylans (HX), which include glucuronoxylans (GX), arabinoglucuronoxylans (AGX), glucurono(arabino)xylans (GAX), arabinoxylans (AX), and complex heteroxylans (CHX). X with  $\beta(1\rightarrow3)$  glycosidic linkages are known to substitute cellulose in the cell wall architecture of green algae (*Caulerpa* sp.), whereas X with mixed  $\beta(1\rightarrow3$  and  $1\rightarrow4)$  glycosidic linkages (Xm) are known cell wall components of red seaweeds of the *Palmariales* and *Nemaliales* [4]. The occurrence of X in higher plants is rather rare. GX with a side chain on the 2-position of either  $\alpha$ -D-glucuronic acid or its 4-O-methyl derivative (Fig. 4.2), occur in hardwood, fruits, and storage tissues such as the pericarp seed of the pear *Opuntia ficus-indica*, luffa (*Luffa cylindrica*) fruits fibers, date seed fibers (*Phoenix dactylifera*), sugar beet pulp, grape skin, and hulls of Jojoba (*Simmondsia chinensis*), and various dicotyls such as ground nut shells, jute baste and bark, sunflower hulls, flax fiber, medicinal plants, kenaf, olive pits, rape stem, red gram husk, and jute bast fiber. Both AGX and GAX have single 4-O-methyl-D-glucuronic acid (MeGlcA) and  $\alpha$ -L-Araf residues attached at position 2 and 3, respectively, to the  $\beta(1\rightarrow4)$ -D-xylopyranose backbone (Fig. 4.3), which might also be slightly acetylated. AGX type occurs in appreciable quantity in coniferous species but not as the dominant hemicellulose components [1]. An exception is the hemicelluloses of the tropic conifer *Podocarpus lambertii* [12], which contains

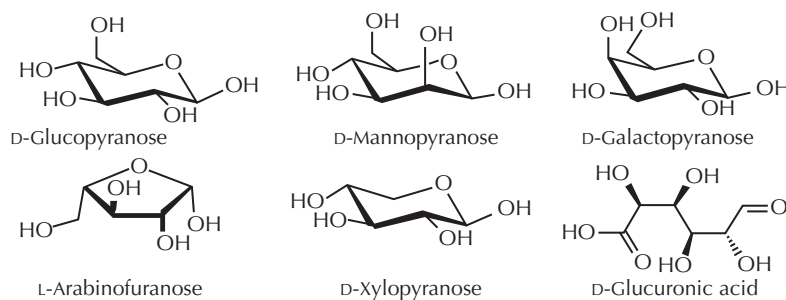


FIGURE 4.1 Main constituents of hemicelluloses.

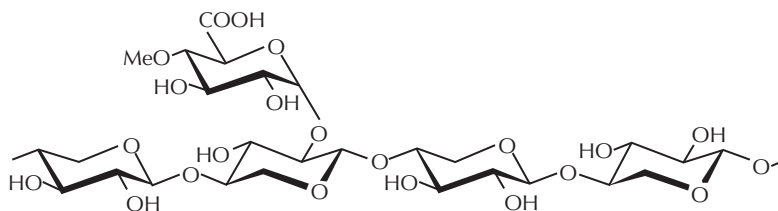


FIGURE 4.2 Primary structure of 4-O-methyl-D-glucurono-D-xylan (MGX).

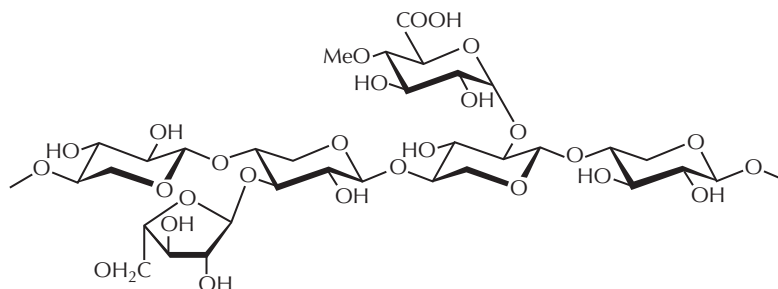


FIGURE 4.3 Primary structure of L-arabino-4-O-methyl-D-glucurono-D-xylan (AGX).

an about equal proportion of mannans and xylans. AGX backbone is more heavily substituted by MeGlcA than that of the hardwood 4-O-methyl-D-glucurono-D-xylan (MGX), with 5–6 Xyl residues per uronic acid group in the former and 10 on average in the latter. AGX are also the dominant hemicelluloses in the cell walls of lignified supporting tissues of grasses and cereals. They were isolated from sisal, CCs, and the straw from various wheat species. In contrast to AGX, the GAX consist of an AX backbone, which contains about 10 times fewer uronic acid side chains than  $\alpha$ -L-Araf ones, and has some Xylp residues doubly substituted with these sugar. The degree of substitution (DS) of GAX appears to vary with the source from which they are extracted. These differences are reflected in the ratio of Ara to Xyl, the content of MeGlcA, and the presence of disaccharide side chain [ $\beta$ -D-Xylp-(1 $\rightarrow$ 2)- $\alpha$ -L-Araf-(1 $\rightarrow$ 3)], as well as the dimeric arabinosyl side chains [ $\alpha$ -L-Araf-(1 $\rightarrow$ 3)- $\alpha$ -L-Araf-(1 $\rightarrow$ 4)] [1, 4]. GAX are located in the nonendospermic tissues of cereal grains such as wheat, corn, and rice bran. AX has been identified in a variety of the main commercial cereal grains: wheat, rye, barley, oat, rice, and corn, sorghum as well as in other plants such as rye grass, bamboo shoots, and pangola grass.

They represent the major hemicellulose component of cell walls of the starchy endosperm (flour) and outer layers (bran) of the cereal grain. AX has a linear backbone that is, in part, substituted by  $\alpha$ -L-Araf residues positioned either on O-3 or O-2 (monosubstitution) or on both O-2 and O-3 (disubstitution) of the Xylp monomer units (Fig. 4.4). In addition, phenolic acids such as ferulic and coumaric acid have been found to be esterified to O-5 of some Araf residues in AX [4, 13]. The CHX are present in cereals, seeds, gum exudates, and mucilages [1]. They are a (1 $\rightarrow$ 4)- $\beta$ -D-xylopyranose backbone decorated, except of the single uronic acid and arabinosyl residues with various mono- and oligoglycosyl side chains. Several CHX samples were isolated from the leaves and barks of tropical dicots such as the *Litsea* species [14]. In a word, xylans are, thus, available in huge and replenishable amounts as byproducts from forestry, agriculture, wood, and pulp and paper industries. The diversity and complexity of xylans mean that many useful byproducts can be potentially produced and, therefore, these polysaccharides are considered as possible biopolymer raw materials for various exploitations.

Two types of mannans exist, namely, galactomannans consisting of  $\beta$ (1 $\rightarrow$ 4) linked D-mannopyranoses and

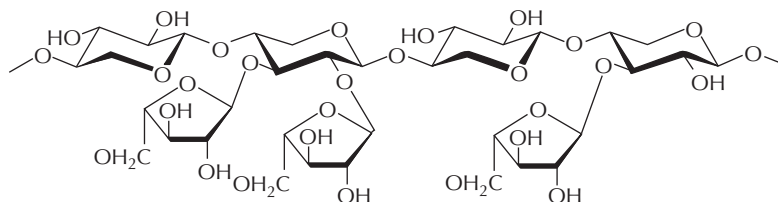


FIGURE 4.4 Primary structure of water-soluble L-arabino-D-xylan (AX).

glucomannans comprised of D-mannopyranose and D-glucopyranose with  $\beta(1\rightarrow4)$  linkages. Both types of mannoglycans have varying degrees of branching with D-galactopyranose residues in the 6-position of the mannose backbone. They mainly occur in softwoods, while minor amounts occur in hardwoods and coffee beans as well as seeds.  $\beta$ -glucans have a D-glucopyranose backbone with mixed  $\beta$  linkages ( $1\rightarrow3$ ,  $1\rightarrow4$ ) in different ratios and can form highly viscous solutions and gels [11]. The mixed-linkage ( $1\rightarrow3$ ,  $1\rightarrow4$ )- $\beta$ -D-glucans ( $\beta$ -glucans) are unique to the Poales, which includes the cereal grasses.  $\beta$ -glucans, as the hemicellulose components of cereal grains, are located in the subaleurone and endospermic cell walls [15–17]. They have been reported to be present in nonendospermic tissues of gramineous monocotyl plants as well [18].

XG have a backbone of  $\beta(1\rightarrow4)$  linked D-glycopyranose residues with a distribution of D-xylopyranose in position 6. The distribution of the side chains divides the XG in two categories: one with two xylopyranose units followed by two glucopyranose units (termed XXGG) and one with three xylopyranose units followed by a single glucopyranose unit (termed XXXG). Additionally, a number of side chains occur on XG, including  $\alpha$ -L-arabinofuranose, which makes the characterization of this group of hemicelluloses especially challenging [11]. XG is a hemicellulosic polysaccharide found in all higher plants, such as dicotyledonous angiosperms, grass, onion, fir tree, olive fruit, soybean, and so on.

#### 4.2.2 Occurrence, Nature, and Classification of Straw Hemicelluloses

It is well known that the most promising dominant sources of xylan exist in many types of agricultural crops such as straw, sorghum, sugarcane, corn stalks and cobs, hulls and husks from starch production. Xylan-rich hemicelluloses constitute an important part of plant cell wall. Xylans, together with other polysaccharides, build up the cell walls of cereal tissues such as grain tissues and straw tissues, and thus become part of the skeletal framework that maintains tissue integrity [19]. One function of xylan is to transport dissolved metabolites and nutrients through the porous hydrated molecular network they establish around the cellulose crystallites. The other is to form intermolecular alignment between polymer chains or noncovalent interactions of xylans with other polysaccharides ( $\beta$ -glucan, cellulose) and, therefore, form multicomponent gels in the complex matrix of the cell walls. In addition, the presence of ferulic acid (FA) residues on the AX chains provides some possibility for covalent polysaccharide polysaccharide or polysaccharide protein interactions [20]. Differences in the molecular features of AX (degree of branching, spatial arrangement of arabinosyl substituents along the xylan backbone, or FA content) could alter the viscoelastic

properties of the gels and hence the resilience, strength, and porosity of the wall matrix. Another potential function of xylans in cell walls of cereal grains is the inhibition of intercellular ice formation, ensuring winter survival of cereals [21]; this effect could be attributed to the enhancement of viscosity and mechanical interference of the AX gel network to the propagation of ice.

As per earlier review articles [11], the variety of sugar residues of hemicelluloses from grasses and cereals is smaller, of which D-xylose, L-arabinose, D-glucose, and D-galactose are the most common. However, in contrast to wood hemicelluloses, however, there is a great variety of linkages and abundance of branching types in graminaceous hemicelluloses, depending on the species of the tissue within a single species, as well as on the age of the tissue [22]. In generally, annual crops (straw, stalks, husk, hulls, bran, etc.) consist of 25–50% hemicelluloses, which generally are classified into AGX, GAX, AX, and CHX based on xylan-type polysaccharides. AGX types containing side chains of 2-O-linked  $\alpha$ -D-glucopyranosyl uronic acid units and/or its 4-O-methyl derivative and 3-linked  $\alpha$ -L-arabinofuranosyl units are typical for lignified tissues of grasses and crop straw. For example, hemicelluloses from CC and the straw from various wheat species mainly are AGX. Wheat straw hemicelluloses are typically L-arabino-(4-O-methyl-D-glucurono)-D-xylan (AGX). The  $\beta(1\rightarrow4)$ -D-xylopyranose backbone is substituted by  $\alpha$ -D-glucuronic acid, mainly MeGlcA at C-2, and L-arabinofuranosyl and D-xylopyranosyl groups attached at C-3. Galactose and xylose residues are potentially linked to arabinofuranosyl branches [3]. Hemicelluloses in maize stems and rye straw are also mainly composed of a xylan structure (AGX), whereas hemicelluloses from rice straw were mainly composed of  $\alpha$ -glucan and AGX [23, 24]. Moreover, L-arabino-(4-O-methyl-D-glucurono)-D-xylan also existed in hemicelluloses from barley straw, and sugarcane bagasse (SCB), which was confirmed in previously published articles [25–27].

AX with Xylp residues substituted at position 3 and/or at both position 2 and 3 of Xyltp by  $\alpha$ -L-Araf units, represent the main xylan component of cereal grains. AX contents vary from 0.15% in rice endosperm to ~13% in whole grain flour from barley and rye, and up to 30% in wheat bran [4, 14]. According to DS, which affects solubility, AXs have been divided into two or three groups, depending on the isolation and fractionation procedures used as well as the distribution patterns [28]. In most cases, the first group includes water-insoluble monosubstituted AXs (Ara:Xyl up to ~0.2–0.3) with  $\alpha$ -L-Araf mainly attached at position 3 of the xylan backbone. The second and third groups comprise water-soluble xylans with a ratio of Ara to Xyl between 0.3 and 1.2 [29]. AXs of the second group with intermediate ratio of Ara to Xyl (0.5–0.9) have shorter sequences of disubstituted Xylp residues than that of the third group. GAX are located in the nonendospermic



tissues of cereal grain such as sorghum wheat, corn, and rice bran. GAX differs from the nonendospermic AGX by containing considerable amounts of 2-linked glucopyranosyl uronic acid (GA) residues. The sorghum GAXs have structural features of AX and contain, in addition, disaccharide moieties composed of 2-*O*- $\alpha$ -L-arabinofuranosyl-L-arabinofuranose linked at position *O*-3 to the xylan backbone [11]. A recent study on wheat bran GAX revealed the presence of lowly and highly substituted GAX fractions, which greatly differ in the amount and type of substitution by the Araf units, but not in the content of the glucuronic acid, half of which occur as the 4-*O*-methyl ether [30]. Feruloylated GAX fractions were isolated from wheat bran by treatment with cold water, steam, and dilute alkali. In addition, CHX can be found in the outer layers (bran) of the cereal grain such as corn bran [31].

Moreover, xylans obtained from grasses, which contain X, AGX, and CHX, have the same backbone as the wood xylans. However, they contain smaller proportions of uronic acids but are more heavily branched and contain larger proportions of L-arabinofuranosyl units [32]. The xylose backbone in grasses is substituted with single residues of  $\alpha$ -D-glucuronic acid (GlcA) and/or 4-*O*-methyl- $\alpha$ -D-glucuronic acid (4-*O*-Me-GlcA) attached at *O*-2 in one out of every 6–12 xylosyl residues [33]. Arabinofuranosyl units are attached to some C-3 position of the main xylan chain. Arabinofuranose units substituted by FA at the *O*-5 position are a widespread component of grass cell walls [4]. The fact that the arabinose units generally seem to be furanosidically linked as terminal groups make them particularly sensitive to acid hydrolysis [34, 35]. The hemicelluloses isolated from crop straws are largely represented as complex heteropolysaccharides whose structure varies in the nature and degree of branching of the  $\beta$ -1,4-linked xylopyranosyl main chain. The chain may be linear, but it is often branched and usually has other glycosidically bound sugar units. Some xylan chains have D-glucopyranosyluronic acid units attached, but the most important acidic hemicelluloses are L-arabino-(4-*O*-methyl-D-glucurono) xylans. The arabinose and uronic acid xylans of these straw hemicelluloses vary considerably from the cereal grain AX, which have an arabinose:xylose ratio of about 1:1 to esparto xylan which has no arabinose at all. During botanical aging, the percentage of side chains on xylans decreases markedly [36], hence straw xylans are found to have a relatively lower arabinose content as compared with the xylans from cereal grains [37]. In the primary cell wall of straw the 4-*O*-methyl-D-glucuronic side chains seems to be absent [38]. However, oligomeric side chains containing other glycosyl residues (e.g., galactose) are also found. An acid galactoarabinoxylan has been isolated from the cell wall of Gramineae by Buchala et al. [39]. It is to be noted that cereal straw are partially acetylated and lose these substituents under alkaline extraction conditions, whereas they were partially preserved under steaming or hot water treatment conditions [40].

## 4.3 ISOLATION, ANALYSIS, AND STRUCTURE

Hemicelluloses are a large group of polysaccharides found in the primary and secondary cell walls of all land and fresh water plants, and in some seaweed. In this section, the methods of isolating hemicelluloses from straw will be reviewed based on the previous researches and new studies. Moreover, the suitable analytical techniques for the determination of structural features will be highlighted in this review. Attention will also be paid to the molecular structure of hemicelluloses from straw.

### 4.3.1 Isolation

Generally, potential resources of hemicelluloses can be prepared by extraction of plant materials, including wood and nonwood plant raw materials, such as agricultural residues, cereal straw, SCB, bamboo, flax straw, industrial hemp, jute, sisal, abaca, reeds, grasses, and other fiber crops. Alternatively, they can also be prepared by extraction of the byproducts of various technologies of wood and processing of annual plants, for example, the hemicelluloses from the viscose process of the rayon fiber technology [41], the xylitol production [42] from process water of pulp by membrane technology [43, 44], and organosolv pulping, heat fractionation process, and paper pulp [5].

Hemicelluloses are the most complex components in the cell wall of wood, straw, and grasses. They form covalent bonds (mainly  $\alpha$ -benzyl ether linkages) with lignin and ester linkage with acetyl units and hydroxycinnamic acids, which restrict the liberation of hemicelluloses from the cell wall matrix. Also the extensive hydrogen bonding between the individual polysaccharide cell wall components may impede the isolation of hemicellulose component [4]. Based on the recent 10 years study, there have been many methods to isolate hemicelluloses from the cell wall of agriculture residues such as cereal straw and SCB. The methods include alkaline extraction, alkaline peroxide extraction, and steam explosion extraction, and so on. However, there is no appropriate method to liberate 100% yield of hemicelluloses without any other components from the cell wall as yet, and hemicelluloses occur to degrade to some extent during the isolation. However, a lot of effort has been put on developing effective procedures to isolate pure and high yield hemicelluloses from agriculture residues. Details of the method are reviewed as follows.

#### 4.3.1.1 Hemicelluloses Isolated by Chemical Reagents

The suitable extraction procedure for the potential commercial hemicelluloses production from straw is still under investigation. It is very important to determine which hemicelluloses type will be produced, what properties

should be expected, and which applications will be applied. And it is also important to propose the further fate (treatment) of the plant fiber residue after the extraction. Various multistep and two-step extraction procedures have been proposed for the isolation of hemicelluloses. Hemicelluloses can be removed from the original or the delignified tissue by extraction with aqueous alkali or, less frequently, with water. For quantitative isolation of hemicelluloses from straw, the typical procedure is as follows: Firstly, the material must be preextracted, preferably with ethanol toluene (2/1, v/v) to remove all lipophilic and hydrophilic nonstructural components. Secondly, the material is delignified, after which the resulting holocellulose is extracted with alkali. Usually, the delignification is affected by chlorine [1], chlorine dioxide [45], or peroxyacetic acid [46]. No completely satisfying method for preparation of holocellulose has been established. Some loss of the hydroxycinnamic acid appendices and degradation of the hemicelluloses appears to be inevitable. In some methods, to avoid saponification of ester linkages, the holocellulose is extracted in succession with dimethylsulphoxide and water. Because the chemical yield obtained by using this procedure is rather low, which seldom exceeds 50%, most commercial hemicelluloses are usually extracted with aqueous alkali. But once the product is obtained, it would be quite similar to the native polysaccharide [47] except for the fact that acetyl, feruloyl-, and *p*-coumaroyl appendices have been saponified.

The isolation of hemicelluloses from straw by aqueous alkali has been widely accepted. Several aqueous alkali processing approaches have been established. One approach is to isolate the hemicelluloses from holocellulose by extraction using aqueous alkali. The hemicelluloses isolated by this method show a light brown color and contain a relatively smaller amount of bound lignin (1–2%). This method was developed for characterization purpose, but it can also be used as a preparative method [48]. The isolation carried out by a gradient extraction using different concentrated alkali solutions yields a crude fractionation of the hemicelluloses [49]. Then, the soluble hemicelluloses

fractions are precipitated with acidified ethanol. In addition, borohydride can be added to minimize the degradation of the reducing end groups. But the cleavage of *O*-acetyl groups cannot be avoided [50] because the pH of the solution has been at 10, and all acetyl groups are split off. The cleavage of the *O*-acetyl groups significantly reduces the solubility of the hemicelluloses in water. Recently, the effects of experimental conditions and alkali type on the yield and composition of xylan-rich hemicelluloses from holocellulose of wheat straw [51–54], sugar beet pulp [55], SCB [56], and rye straw [57] have been extensively investigated. The extractability of hemicelluloses from annual plants is easier than that of wood hemicelluloses because of the lower amounts and different structure of lignin. It can be affected by the alkali type and isolation conditions and improved by a multistep mechanical-chemical treatment [51]. The effect of the different type alkali on sugar composition of hemicelluloses from wheat straw holocellulose was summarized in Table 4.1. Usually, aqueous solutions of potassium, sodium, and lithium hydroxide are appropriate to be used to isolate hemicelluloses from straw, but the preferred alkali was potassium hydroxide mainly because the potassium acetate formed during the neutralization of the alkali extract is more soluble in the alcohol used for precipitation than sodium acetate [51]. The optimal time for extracting hemicelluloses using 10% KOH and 2% H<sub>3</sub>BO<sub>3</sub> at 20 °C was found to be between 21 and 26 h. The suitable concentrations of KOH for extracting hemicelluloses at 20 °C for 2 h were between 20% and 30%, which yielded hemicelluloses from 34.21% to 34.93%. The optimum extraction concentration of H<sub>3</sub>BO<sub>3</sub> in the extraction of 24% KOH at 20 °C for 2 h was either 2 or 5%. The molar ratios of xylose:arabinose:galactose:glucose in 24% KOH and 2% H<sub>3</sub>BO<sub>3</sub> for 2 h at 20 °C extracted hemicelluloses were 58:5.6:0.7:2.0, and in that the content of uronic acid was 3.1%. The average *M<sub>w</sub>* of the hemicelluloses was about 12 000 g·mol<sup>−1</sup>, and the total content of phenolic acids and aldehydes in hemicellulose extracted was 0.8%. Furthermore, the addition of boric acid or borate also has the ability to markedly enhance

**TABLE 4.1** Sugar Composition (relative %) of Hemicelluloses (Adapted from [51])

Chemical concentration	Arabinose	Xylose	Mannose	Galactose	Glucose	Yield (%)
15% Ca(OH) <sub>2</sub> + 2% H <sub>3</sub> BO <sub>3</sub>	15.6	77.3		1.9	4.3	5.46
15%NH <sub>3</sub> + 2% H <sub>3</sub> BO <sub>3</sub>	7.4	87.9		1.3	3.3	17.84
15% KOH +2% H <sub>3</sub> BO <sub>3</sub>	10.4	82.6		2.2	5.0	33.59
15% NaOH + 2% H <sub>3</sub> BO <sub>3</sub>	7.5	86.4	Tr	2.1	4.0	34.80
15% LiOH + 2% H <sub>3</sub> BO <sub>3</sub>	7.8	87.0	Tr	2.2	3.9	35.08

Tr, trace.

Hemicelluloses extracted from wheat straw holocellulose using various alkalis at 20 °C for 2 h.

the dissolving ability of alkaline solvents toward certain classes of polysaccharides, especially for glucomannans, mainly because of the formation of borate complexes with hydroxyl groups in the *cis* position [58]. The 'optimum extraction concentration' of boric acid is either 2 or 5%, depending on which of the following criteria is considered most important: consumption of boric acid or total hemicellulose yield.

Another method of these aqueous alkali processing approaches is to isolate hemicelluloses from the dewaxed straw directly. Hemicelluloses and lignin could be obtained by adjusting the pH of the solution obtained after alkali extraction. This procedure is an eco-friendly extraction process. Thus, recently, this procedure has also been used in the isolation of hemicelluloses from wheat straw [3, 59], sugar beet pulp [60], barley straw [61], maize stems, rye straws, and rice straw [23]. It was found that the treatment of dewaxed maize stems, rye straw, and rice straw with 1 M sodium hydroxide (NaOH) at 30 °C for 18 h resulted in a dissolution of 78.0, 68.8, and 82.1% of the original lignin, and 72.1, 72.6, and 84.6% of the original hemicelluloses, respectively. This high solubility of lignin and hemicelluloses was undoubtedly because of the cleavage of the ester bonds between hydroxycinnamic acids, such as *p*-coumaric and FAs, and lignin or hemicelluloses, and the  $\alpha$ -benzyl ether linkages between lignin and hemicelluloses from the cell walls by alkali. Additionally, it was also found that the treatment with 1 M NaOH under the same condition removed 89.2, 92.6, and 88.9% of the original silica from maize stems, rye straw, and rice straw, respectively. As the silica is concentrated in the outer parts of cereal straw or stems, it is very likely that treatment with selected chemicals, such as 1 M NaOH, may be very effective for desilication from cereal straw. Meanwhile, the treatment also solubilized significant amounts of protein and noticeable quantities of low *M<sub>w</sub>* polysaccharides from the straws and stems. However, because of the lignin hemicellulosic complex in the cell walls of straw, the content of phenolics in the hemicellulosic fractions extracted directly from dewaxed straw was 5–10 times higher than that in the hemicellulosic fractions extracted from straw holocelluloses. This indicates that the hemicelluloses obtained by this process had higher amount of lignin than the hemicelluloses obtained by the alkali extraction from holocellulose straw.

Recently, it has been reported that alkaline peroxide is an effective agent for both delignification and solubilization of hemicelluloses from straws and grasses [61]. It is, generally, accepted that hydroperoxide anion ( $\text{HOO}^-$ ) formed in alkaline media, is the principal active groups in hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) bleaching systems. In contrast,  $\text{H}_2\text{O}_2$  is unstable in alkaline conditions and readily decompose into hydroxyl radicals ( $\text{HO}\cdot$ ) and superoxide anion radicals ( $\text{O}_2^{\cdot-}$ ). This is particularly true in the presence of certain transition metals such as manganese, iron, and copper. These radicals are thought to cause the

oxidation of lignin structures which lead to the introduction of hydrophilic (carboxyl) groups, cleavage of some interunit bonds and, eventually, the dissolution of lignin and hemicelluloses [62]. As a comparison, treatment of rye straw with a dilute alkaline solution at pH 11.5 for 12 h at 50 °C in the absence of  $\text{H}_2\text{O}_2$  yielded only 7.3% of the original hemicelluloses, whereas 69.6% original hemicelluloses could be obtained in the presence of 2%  $\text{H}_2\text{O}_2$  under the same condition [63]. Moreover, the yield of hemicelluloses from maize stems treated by using 2%  $\text{H}_2\text{O}_2$  at 45 °C for 12 h at pH 11.5–12.0 was higher, which was up to 63.3–64.7% of the original hemicelluloses, and the weight-average molar weight was between 69 060 and 54 740  $\text{g}\cdot\text{mol}^{-1}$ , which was higher than those obtained by organic acid such as peroxymonosulfuric acid and peracetic acid [64, 65]. Sun et al. also found that more than 80% of the original hemicelluloses and over 90% of the original lignin was solubilized during the treatment of cereal straws such as wheat, barley, rice, oat, rye straw, and maize stems with 2%  $\text{H}_2\text{O}_2$  at 48 °C for 16 h at pH 12.0–12.5 (Table 4.2). These hemicellulose preparations were white in color and contained much smaller amount of associated lignin (3–5%) than those obtained from the traditional alkali extraction process [66]. To gain maximum dissolution of the hemicelluloses, it was not necessary to continuously regulate the reaction pH, even though over the course of the treatment (48 °C, 16 h) the reaction pH increased from 12.0–12.5 to 12.9–13.1. As the reaction pH became more alkaline, increasing amounts of hemicelluloses were solubilized, and the yield of the residue decreased. Incremental increase of the initial reaction pH from 11.5 to 12.5 resulted in an increase of the dissolution of hemicelluloses by about 20%. For the isolation of hemicelluloses from straw, in particular, two-step procedures with a NaOH/ $\text{H}_2\text{O}_2$  delignification step were shown to be more acceptable in practice than the hazardous delignification with sodium chlorite. The typical two-step procedure with a NaOH/ $\text{H}_2\text{O}_2$  delignification step to isolate hemicelluloses from straw is shown in Fig. 4.5. The two-stage treatment resulted in a solution containing 88.5% of the original hemicelluloses from rice straw by the first extraction with 1% NaOH at 55 °C for 2 h and, then, following treatment with 5.0%  $\text{H}_2\text{O}_2$  at 45 °C for 12 h at pH 11.5 [67]. In addition, the comparison of these hemicellulosic preparations indicated that the alkali treatment in the absence of  $\text{H}_2\text{O}_2$  was favored to the solubilization of the small molecular size hemicelluloses, which are rich in glucose, probably originating from  $\alpha$ -glucan, whereas the second stage treatment by alkaline peroxide enhanced the dissolution of larger molecular size hemicelluloses, which are rich in xylose, principally resulting from L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan. Another typical two-step procedure with an ethanol  $\text{H}_2\text{O}/\text{H}_2\text{O}_2$  step by the treatment with the ethanol  $\text{H}_2\text{O}$  (60/40, v/v) under acid catalyst (0.2 N HCl) at 70 °C for 4 h and following the

**TABLE 4.2** The Yield, Neutral Sugar Composition, and Content of Uronic Acid and Lignin of Hemicelluloses Obtained by Treatment of Wheat, Rice, Rye, Barley, and Oat Straw, and Maize Stems with 2% H<sub>2</sub>O<sub>2</sub> at 48 °C for 16 h at pH 12.2, Respectively (Adapted from [66])

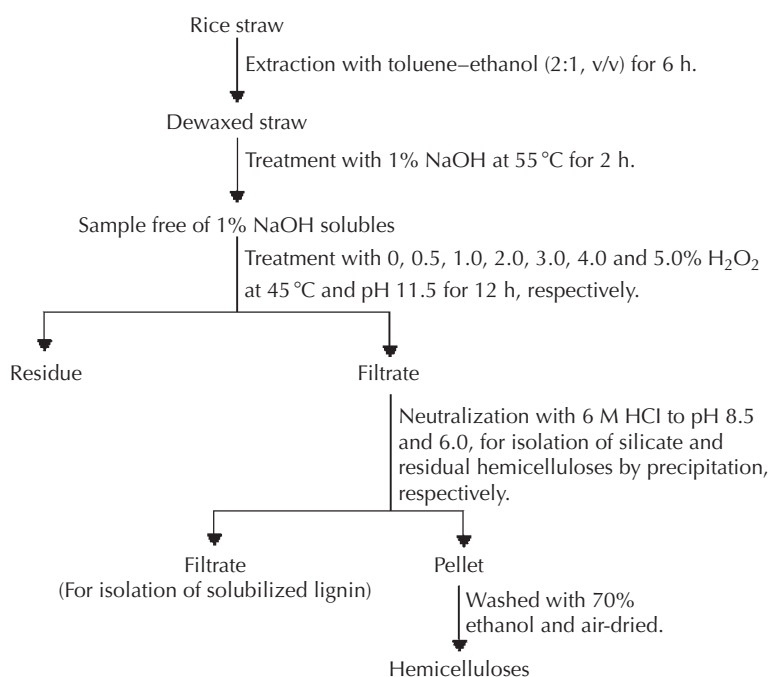
Cereal straw/ stems	Yield (%) <sup>a</sup>	Neutral sugar composition (%) <sup>b</sup>							Uronic acids (%) <sup>b</sup>	Lignin content (%) <sup>b</sup>
		Rha	Fuc	Ara	Xyl	Man	Glc	Gal		
Wheat	29.6	0.8	ND <sup>c</sup>	13.8	60.5	0.4	9.8	4.5	4.9	5.1
Rice	22.3	0.6	0.3	14.9	56.3	ND	22.3	4.8	4.3	4.7
Rye	26.6	0.5	ND	11.2	65.3	0.3	6.1	3.3	8.1	4.8
Oat	25.6	0.4	ND	10.8	68.3	0.3	6.4	3.6	5.5	4.3
Barley	23.3	0.4	ND	10.6	66.1	0.5	7.6	3.7	5.8	4.5
Maize	22.7	0.5	0.3	15.2	62.7	0.8	6.3	4.7	5.3	3.7

Note: Ara, arabinose; Xyl, xylose; Man, mannose; Rha, rhamnose; Fuc, fucose; Glc, glucose; Gal, galactose.

<sup>a</sup>Percent dry matter (w/w).

<sup>b</sup>Percent hemicelluloses (w/w).

<sup>c</sup>ND, not detectable.



**FIGURE 4.5** The typical two step procedure with a NaOH/H<sub>2</sub>O<sub>2</sub> delignification step to isolate hemicelluloses from straw [67].

treatment with 2% H<sub>2</sub>O<sub>2</sub> at pH 11.5 for 16 h at 45 °C to treat rice straw resulted in the yield up to 88.2% of the original hemicelluloses from rice straw [68]. Therefore, the two-step treating process minimized the use of H<sub>2</sub>O<sub>2</sub> and allowed the use of more extreme extraction conditions to increase the yields of hemicelluloses, and this two-step treating process also could release large molecular mass of hemicelluloses. Furthermore, milder extraction conditions and even oxidant-free dilute alkalis can be used to yield more than 80% of the xylan component of CCs, wheat straw,

barley straw, rice straw, rye straws, maize stems, and SCB [25, 26, 67–73]. Additionally, some assistant agent can improve the treatment condition using H<sub>2</sub>O<sub>2</sub>. To improve the color of the isolated hemicelluloses, the products were subjected to posttreatment with H<sub>2</sub>O<sub>2</sub> that was activated with tetraacetylenediamine (TAED) [74, 75] and cyanamide [76]. Additionally, the TAED-activated peroxide posttreatment cleaved most of the linkages between lignin and hemicelluloses as shown by a relatively lower content of associated lignin (2.5–5.0%) in the polymers [74]. It was



found that the cyanamide-activated  $\text{H}_2\text{O}_2$  posttreatment, under the conditions given, did not result in a substantial oxidation of the residual hemicellulosic polymers [76].

Organosolv treatment is another potential method in the isolation of hemicelluloses. In recent years, the hazardous and expensive  $\text{NaClO}$  delignification step is substituted by the procedure of aqueous organosolv treatment, which yields xylan-rich polysaccharide fractions contaminated to various extents with lignin and degradation products of the cell wall components [64, 77]. However, the yield of the prepared hemicelluloses was lower in comparison to those obtained from partially delignified straw meal. In this field, organosolv treatment (based on the utilization of organic solvents as delignification media) allows the fractionation of the lignocellulosic substrates to give chlorine-free lignin, cellulose, and valuable hemicelluloses degradation products, providing a superior approach from the point of view of the biomass utilization. The easy recovery of solvent and the efficient pollution control are important features of these kinds of processes [78]. It has been performed with a great variety of solvents using acid (mineral, organic, or Lewis acids) and either alkaline or acid catalyst [79, 80]. For example, in the acetosolv process, the pulping medium was made up of concentrated acetic acid, and  $\text{HCl}$  was added as a catalyst to promote the hydrolytic degradation of lignin and hemicelluloses under milder operation conditions [81]. Xu and coworkers investigated comparably the influence of various organosolv treatments, such as acetic acid  $\text{H}_2\text{O}$  (65/35, v/v), acetic acid  $\text{H}_2\text{O}$  (80/20, v/v), acetic acid  $\text{H}_2\text{O}$  (90/10, v/v), formic acid acetic acid  $\text{H}_2\text{O}$  (20/60/20, v/v/v), formic acid acetic acid  $\text{H}_2\text{O}$  (30/60/10, v/v/v), methanol  $\text{H}_2\text{O}$  (60/40, v/v), and ethanol  $\text{H}_2\text{O}$  (60/40, v/v) using 0.1%  $\text{HCl}$  as a catalyst at 85 °C for 4 h, on characterization of hemicelluloses from the wheat straw [82]. The optimum condition for degradation of hemicelluloses was found to use a mixture of formic acid acetic acid  $\text{H}_2\text{O}$  (30/60/10, v/v/v), which yielded 76.5% of the original hemicelluloses from wheat straw. In comparison, the degraded hemicellulosic preparations isolated during the treatment with organic acids were more linear and partially acetylated, whereas the two acidic alcohol-degradable hemicelluloses were more branched. In addition, the organosolv treatments under acidic condition could get the lower  $M_w$  ranging between 8480 and 18 940  $\text{g mol}^{-1}$  of degraded hemicelluloses. The similar results were also confirmed by the isolation of SCB hemicelluloses with the acidic 1,4-dioxane posttreatment and alkali pretreatment [83]. Compared with the alkali-soluble hemicelluloses, acidic organic-solvent degraded hemicelluloses are more branched and have lower  $M_w$  and lower thermal stability. It meant that substantial degradation of hemicellulosic polymers occurred during the acidic organic solvent treatment. Furthermore, Sun and his coworkers investigated the isolation methods of wheat straw hemicelluloses, which included organosolv pretreatment and  $\text{H}_2\text{O}_2$  treatment [76], and also found that the

hemicelluloses were sequentially extracted with high yield/purity, using acidic dioxane/water solution and dimethyl sulfoxide, from ball-milled wheat straw [84]. Therefore, the application of organic acids during the preparation of degraded hemicelluloses and lignin from lignocellulosic materials, such as cereal straw and wood, might result in a better exploitation of the raw materials.

#### 4.3.1.2 Hemicelluloses Isolated by the Combination of Chemical Treatment with Mechanical Treatment

Recently, another potential method for isolation of hemicelluloses has been proposed to combine chemical treatment with mechanical treatment. The mechanical and chemical effect of ultrasonication on the cell wall material during alkaline extraction of annual plants was shown to be very effective. Higher yields of hemicelluloses can be achieved at lower temperatures and shorter extraction times [85]. The application of ultrasound was shown to be very effective during the alkaline extraction of hemicelluloses from CC [70, 85], the seed coat of buckwheat [86], corn hulls [70, 87], wheat straw [88, 89], and SCB [90]. The extractability of the wheat straw hemicelluloses with and without the application of ultrasonic irradiation in 0.5 M  $\text{KOH}$  aqueous solution was explored, and the result showed that ultrasonically assisted extraction in a period of 20–35 min produced a slightly higher yield of hemicelluloses and lignin than those of the classical alkali procedure by 0.8–1.8% of the original hemicelluloses and 0.6–5.3% of the original lignin, respectively [89]. The hemicelluloses, obtained by ultrasound-assisted extraction, seemed more linear and less acidic than the hemicelluloses extracted by alkali in the absence of ultrasonic irradiation. In addition, the hemicellulosic preparations obtained by ultrasound-assisted extractions showed a relatively lower content of associated lignin but a higher  $M_w$  and a slightly higher thermal stability in comparison with the hemicelluloses isolated by alkali without ultrasonic irradiation [62]. There were no substantial differences in the main structural features of hemicellulosic preparations isolated by the classical or ultrasound-assisted extraction procedures. Furthermore, ultrasound was also applied during the alkaline organosolv extraction of wheat straw [91]. Treatment of dewaxed wheat straw with 0.5 M  $\text{NaOH}$  in 60% aqueous methanol at 60 °C for 2.5 h with ultrasonic assistance for 5–35 min resulted in an increasing yield of hemicelluloses by 2.9–9.2% of the original hemicelluloses as compared with the yield of hemicelluloses obtained under the same condition, but without using ultrasonic irradiation. Except for a slightly higher content of xylose, a relatively lower content of associated lignin, a lower  $M_w$ , and a slightly lower thermal stability during the first stage decomposition of the hemicelluloses obtained by ultrasound-assisted extractions. No substantial difference in the



main structural features of the hemicellulosic preparations isolated by the classical or ultrasound-assisted extraction procedures was observed. Therefore, the application of ultrasound treatment during the preparation of hemicelluloses and lignin from agricultural residues and wood samples might result in a better exploitation of the raw material.

The use of an extruder-type twin-screw reactor makes the extraction more feasible [92]. Xylans were coextracted from a mixture of wheat straw and wheat bran by indirect alkali extraction in a twin-screw extruder [93]. The best results of the production including yield and the extraction properties were obtained by using low alkali content. A twin-screw extruder allowed a much lower consumption of chemicals and waste water volumes. Extraction was done with 14 times less consumption of NaOH in the extruder than in the stirred reactor. This technique which combines wheat straw and wheat bran extraction is attractive because it allows valorization of both liquid extract and solid raffinate (agromaterials), and because it is a continuous process, it is good for the treatment of large quantities of organic matter.

#### 4.3.1.3 Hemicelluloses Isolated by Heat Treatments

Recently, it has been known that the disintegration of lignified cell walls of cereal straw and wood can be achieved by steam explosion treatments resulting in solubilization of partially depolymerized hemicelluloses [7, 94, 95]. High-pressure steaming with rapid decompression (explosion) has been claimed as one of the most successful pretreatment methods for fractionating lignocellulosic materials into their three major components and enhancing the susceptibility of cellulose to enzymatic attack. The fractionation of wheat straw was studied by using a two-stage process based on a steam explosion pretreatment followed by alkaline peroxide posttreatment [96]. Straw was steamed at 200 °C for 10 and 33 min and 220 °C for 3, 5, and 8 min. The steamed straw was washed with water to yield a solution rich in hemicelluloses-derived mono- and oligosaccharides (20.5–28.5%) together with small amounts of degraded hemicellulosic polymers (2.4–6.2%) and minor quantities of lignin (1.9–2.1%). The washed fiber was posttreated by 2% alkaline peroxide (pH 11.5) at 50 °C for 5 h. The alkali-soluble lignin (13.7–15.0%) and hemicelluloses (8.4–13.3%) were recovered by precipitation. The posttreatment was sufficient to remove lignin and survived hemicellulosic polymers and increases the brightness of the exploded straw significantly. The two-stage treatments degraded 77.0–87.6% of the total original hemicelluloses and 92.3–99.4% of the total original lignin in total. Furthermore, a combination of alkaline pretreatment and steaming of wheat straw and barley straw was recently proposed by our research group. The purpose of this study presented a method to pretreat wheat and

barley straw for ethanol production and at the same time extract AX in its polymeric form. The straw after spraying NaOH solution for 30 min were steamed at the temperature between 180 °C and 190 °C for the residence time from 5 to 50 min. This study showed that 30% of the xylan in barley straw and 40% of the xylan in wheat straw can be extracted in its polymeric form by using a minor alkaline addition before the steam pretreatment. The fact showed that neither hydroxymethylfurfural (HMF) nor furfural was produced in the pretreatment step, which is a great benefit for the flexibility of a future process. In addition, a method comprised of steam exploding plant straw at steam pressure 1.0–1.5 MPa and straw water content 10–35% for 2–10 min, swelling and washing with 40–60 °C water, adjusting pH of the washing solution to 5.5–6.0, settling for 12–20 h to obtain hemicelluloses was proposed by Chen [97]. It was noted that the 67–80% yields of hemicelluloses could be obtained. An eco-friendly process of wheat straw fractionation by steam explosion coupled with ethanol extraction was studied [98]. The wheat straw was steam exploded for 4.5 min with moisture of 34.0% and a pressure of 1.5 MPa without acid or alkali. It was found that the total recovery rate of hemicelluloses was 80%, and lignin yield was 75% by acid precipitation and 85% ethanol solvent was recovered. The cellulose recovery rate was 94%. Moreover, the steam explosion was also used in the pretreatment of corn stover biomass [99, 100], CC [101], barley straw [102], rice straw [103], wheat straw [104–107] to remove hemicelluloses and lignin to increase the digestion of celluloses in ethanol production.

Hot water could be used as a tool to separate water-soluble hemicelluloses from cell wall straw. A few reports about the comparison of hemicellulosic fractions obtained by hot water and alkaline solution have been investigated [26, 108]. Usually, the treatment to dewaxed straw with hot water was between 55 °C and 80 °C for 1 or 2 h, while the yield of water-soluble hemicelluloses was very low at 5% (based on dry materials). Previous articles reported that the smaller molecular size and more branched hemicelluloses, which are rich in glucose, probably originating from  $\alpha$ -glucan and pectic polysaccharides were extracted by the hot water treatment, whereas the larger molecular size and more linear hemicelluloses were dissolved by the alkali treatment. It could be interpreted that hemicelluloses with the low  $M_w$  and high branch degree were easily dissolved in hot water, whereas hemicelluloses with high  $M_w$  could be dissolved by the alkaline solution treatment. Comparably, hemicelluloses extracted by hot water contained more lignin content than those extracted by alkaline solution.

Besides steam explosion and hot water extraction, other heat treatments like microwave irradiation have also been used for the isolation of hemicelluloses from wheat straw. The microwave-assisted alkali pretreatment of wheat straw for removing hemicelluloses and lignin for ethanol production was brought forward by Zhu [109]. This treatment

involved both physical and chemical methods to extract hemicelluloses. At the same alkali concentration (up to 1%), lower hemicelluloses yield (7.8%) in the residues was obtained after microwave-assisted alkali pretreatment of wheat straw than that yield (11.2%) in the residues after conventional alkali pretreatment. Usually, the treatment temperature and initial pH of the aqueous extraction media had a significant effect on the structure of hemicelluloses and molecular mass. The results of aqueous hemicelluloses extraction performed at different temperatures showed that hemicelluloses recovery could be improved by increasing the temperature of the microwave treatment, but at the same time, the  $M_w$  of the isolated polymers decreases along with the pH of the reaction mixture. Application of NaOH instead of acid in the treatment resulted in considerably higher yields (11%) with longer polysaccharide chains ( $1.3 \times 10^5$ ) [40]. The  $M_w$  of the hemicelluloses isolates by NaOH was greater than those separated by water extraction; however, hemicelluloses yields were far less than those achieved with pure water. Comparing with alkaline extraction, it could be concluded that microwave treatment provided lower  $M_w$  and lower polymer recovery, but on the other hand, it performed well without the application of any chemicals. Therefore, the difficulty with this technology is how to achieve a good yield without extensive degradation of the hemicelluloses and contamination with dissolved lignin and cellulose. However, it could be a novel eco-friendly way of isolation of hemicelluloses.

More recently, the semipilot scale of continuous flow type hydrothermal reactor has been investigated to separate hemicellulose fraction from corncob [110–114]. The effective recovery of hemicellulose using tubular type reactor at 200 °C for 10 min was obtained [114]. From constituent sugar analysis of corncob, 82.2% of xylan fraction was recovered as mixture of xylose, xylooligosaccharides, and higher-xylooligosaccharide with DP more than 10. After purification of solubilized fraction by hydrothermal reaction such as ultrafiltration and ion-exchange resin, higher-xylooligosaccharide was recovered as the precipitate. This precipitate was identified as nonblanched xylan fraction with DP mainly varying from 11 to 21. The moderate hydrothermal reaction process without chemicals such as acid or alkali is thought to be desirable for environmental reasons because it is not necessary to neutralize and desalt in liquid-waste treatment. In addition, it is a remarkable point that the hydrothermal reaction process for xylooligosaccharides production from corncob has been analyzed based on kinetic modeling.

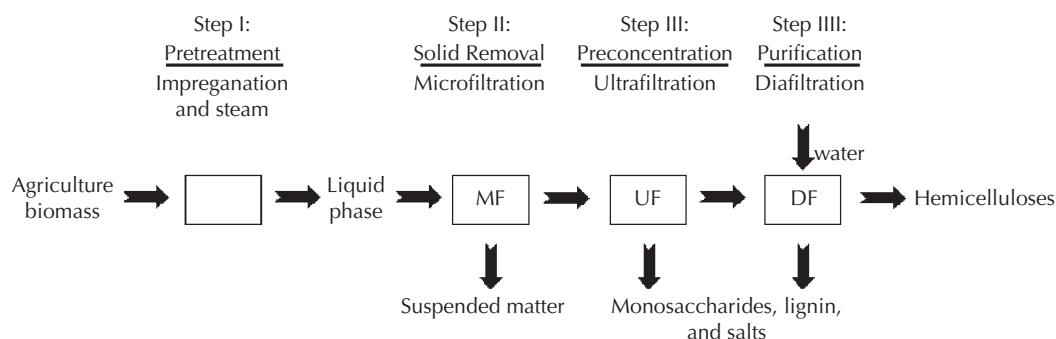
#### 4.3.1.4 Hemicelluloses Isolated by Membrane

Another potential method to extract hemicelluloses from process water and waste streams from thermomechanical pulp of spruce by membrane technology has been extensively discussed recently [43, 44]. Moreover, a new

attempt to use the membrane technology in extraction of hemicelluloses from agriculture biomass such as barley husks and wheat straw and bran has been investigated [93, 115, 116]. The method involves the following four steps: (1) gaining the liquid phase in which hemicelluloses were dissolved after pretreatment of agriculture biomass by steam (2) removal of high molecular species by micro-filtration, (3) preconcentration of hemicelluloses by ultra-filtration (UF), and (4) reduction of the concentration of salts and monosaccharides by diafiltration. This typical process is shown in Fig. 4.6. Membrane filtration provides an appropriate method to divide a feed stream containing two or more components with different sizes into two streams, whereas at least one of the two streams is enriched in one or more components of the feed stream. The membrane thereby acts like a selective barrier, which is permeable for some of the components but impermeable for others. In general, temperature and pressure have effects on the purification of hemicelluloses. Membrane decides the characterization of hemicelluloses isolated, and hydrophobic membranes have severe fouling susceptibility and membranes with cut-off 1 kDa has less efficient separation between hemicelluloses and monosaccharides and salts than membranes with 5 kDa cut-off. Marechal et al. [93] studied the concentration of liquid obtained after the extraction from a mixture of wheat straw and wheat bran by alkali extraction in a twin-screw extruder, which was designed to increase the xylan yield by UF with 50 kDa and 10 kDa membranes before the alcohol precipitation. The membrane configuration and mol. wt. cut off (MWCO) must be adapted to each solution to limit fouling and concentration polarization. A permeate flux of 20 dm<sup>3</sup>/h.m<sup>2</sup> was obtained at a final concentration ratio of 2. UF allows for a partial demineralization of the solution, but it does not change the properties of the final powder. This procedure might be a technical approach to design a high-throughout process.

#### 4.3.1.5 Fractionation and Purification

Precipitation of the polysaccharide fractions by addition of miscible organic solvents to aqueous solutions is one of the main methods of recovery and purification. Ethanol is the most commonly used solvent, but methanol, acetone, and other organic solvents have also been applied for fractionation of hemicelluloses. However, using these solvents, part of the hemicellulosic materials still remains in solution, and usually it can't be recovered. For example, an oat-leaf holocellulose was treated with aqueous acid, and the precipitated hemicelluloses were recovered after the addition of an excess amount of acetone. The solvents were removed from the remaining solution, and after dissolving the potassium acetate in ethanol, the hemicellulosic material recovered accounted for 13.2% of the hemicelluloses extracted [117]. To further understand more structural



**FIGURE 4.6** The procedure of extraction of hemicelluloses from agriculture biomass.

information on fractionated hemicelluloses, the hemicellulosic fractions with different structural features could be subfractionated by means of a gradual ethanol precipitation. Recently, a few researches about the effect of the methods of a graded ethanol precipitation on the hemicellulosic fractions from SCB have been also investigated. Our research group investigated the sequential treatment of dewaxed SCB with hot water and NaOH with 1% and 3% concentration, and also studied the fractions which were subfractionated by gradual precipitation at the ethanol concentrations of 15, 30, and 60% (v/v). It was found that the Ara/Xyl ratio increased with an increase in ethanol concentration from 15 to 60%, and the AX with higher Ara/Xyl ratios had higher  $M_w$ s. It was also found that there was no significant difference in the structural features of the precipitated hemicellulosic subfractions, which are mainly consisted of L-arabino-(4-O-methyl-D-glucurono)-xylan, although the difference may occur in the distribution of branches along the xylan backbone and the  $M_w$ s. Moreover, the fact that increment of Ara/Xyl ratio in fractions precipitated at high alcohol concentration had been verified in the extraction of cereal flour AX by Vieter et al. [118] and Gruppen et al. [119]. But Hoffmann and coworkers [120] did not observe such relationship, while they found the highly branched AX fractions with high Ara/Xyl ratio had higher  $M_w$  than their less branched counterparts. Vieter et al. [118] reported no significant differences in  $M_w$  distributions by high performance size exclusion chromatography (HPSEC) test of fractions with varying Ara/Xyl. Those inconsistencies maybe resulting from differences in the techniques and solvent conditions employed during the measurement of the  $M_w$  [19]. In addition to the neutral or mildly acidified organic solvents, some more specific precipitation agents are also known, e.g., barium hydroxide for glucomannans and cetyltrimethylammonium bromide or hydroxide for glucomannans and glucuronoxylan. Hemicellulosic complexes precipitated by iodine in calcium chloride appear to be relatively unsubstituted by nonxylose residues, whereas the material remaining in solution is more heavily substituted by such residues. The methods used to fractionate the hemicelluloses of straws and grasses are similar to those used to fractionate hemicelluloses from

wood. The hemicelluloses may be fractionated as their acetates by precipitation from solution with ammonium sulfate, or by chromatography on diethylaminoethyl (DEAE) cellulose. The precipitated hemicellulose preparation can, if desired, be further purified by column chromatography [66].

To study the structural difference in the hemicellulosic fractions isolated by sequential extraction and fractionation, our research group has studied the water-soluble and alkali-soluble hemicelluloses obtained from SCB by fractionating them with DEAE-cellulose-52 chromatography. The sequential treatments of dewaxed bagasse with water (55 °C for 9 h) and 1% NaOH (50 °C for 6 h) aqueous solution yielded 16.6% hemicelluloses from bagasse and comprised 49.5% of the original hemicelluloses. Then the water-soluble and alkali-soluble hemicelluloses were subfractionated on DEAE-cellulose-52 chromatography and obtained six hemicellulosic subfractions by eluting with water, 0.1 M and 0.3 M NaCl aqueous solution, respectively. Noticeable differences in the chemical composition and  $M_w$ s were observed among the gradually precipitated hemicellulosic subfractions obtained from the water-soluble and alkali-soluble hemicelluloses. It was found that with increasing concentration of NaCl aqueous solution from 0.0 to 0.3 M, alkali-soluble hemicellulosic subfractions with increasing Ara/Xyl ratio were eluted from 0.07 to 0.16, and the hemicellulosic subfractions with higher Ara/Xyl ratios had higher  $M_w$ s. This indicated that there is the diversity among hemicelluloses present in the cell wall of bagasse, which vary in their degree of branching and possibly in their  $M_w$ . This study also demonstrated that the 0.3 M NaCl-eluted hemicelluloses had a higher  $M_w$  and higher thermal stability than those of the water and 0.1 M NaCl-eluted hemicellulosic fragments. Therefore, these advantages implied that the method of alkaline extraction followed by fractionation by DEAE-cellulose-52 chromatography could be used for isolation of polysaccharides having a different degree of branching and  $M_w$  from renewable materials for industries.

In addition, supercritical antisolvent precipitation is a relatively novel technology, which can be used for controlled preparation of polymer particles from solutions. This is realized by the addition of an antisolvent to a polymer

solution, which causes supersaturation of the polymer, especially under supercritical conditions. The particle size of the precipitates can be adjusted mainly by the rate of supersaturation. Spherical xylan or mannan particles having a narrow particle size distribution were precipitated from hemicellulose solutions in dimethylsulfoxide (DMSO) or DMSO/water mixtures by carbon dioxide as an antisolvent. Based on the type of hemicellulose, the DMSO/H<sub>2</sub>O ratio, and the precipitation conditions such as pressure and temperature, the resulting particle size can be adjusted within a wide range from less than 0.1  $\mu\text{m}$  to more than 5  $\mu\text{m}$ . Nano- and microstructured native xylylans and mannans as obtained can be used in many applications such as encapsulation of active compounds, slow release agents, or chromatographic separation materials [121]. Therefore, this technology will be widely applied in many industries in the future.

Finally, it has to be underlined that a certain xylan-type polysaccharide can be isolated from certain plant sources using the same procedure, and it seems to be possible to produce biopolymers with a similar structure. However, differences may occur in the fine structure of the polymers, that is, in the distribution of the branches along the xylan backbone.

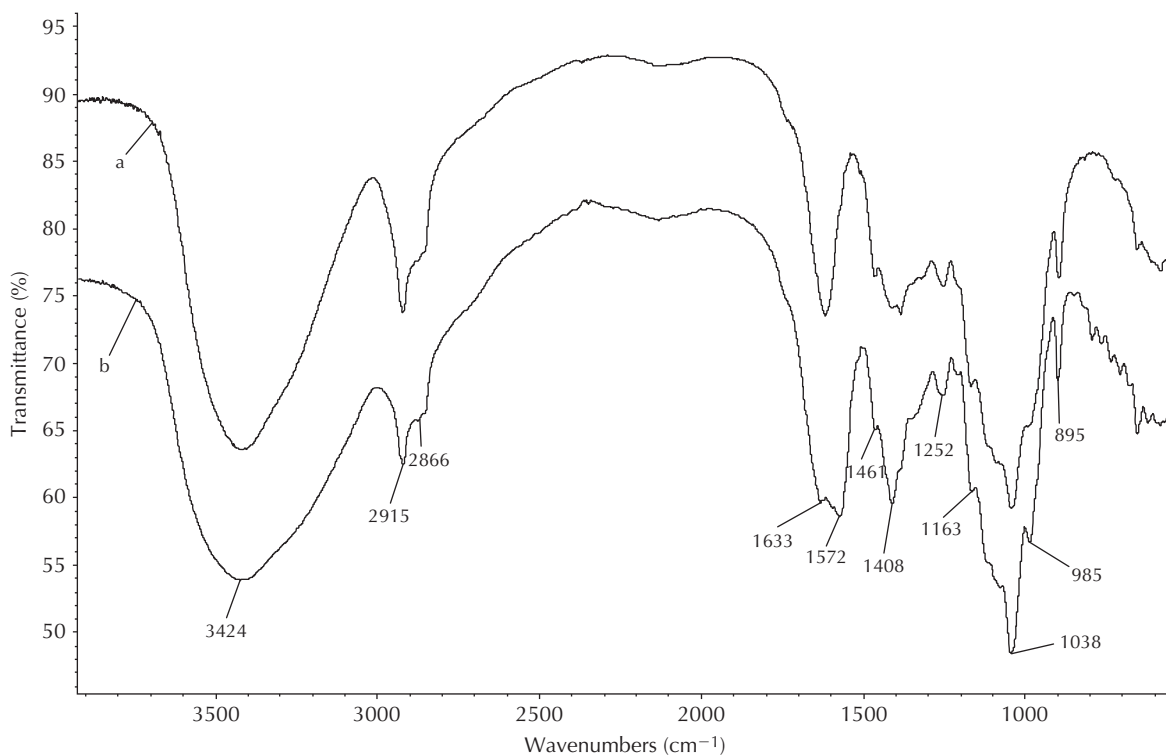
### 4.3.2 Structural Analysis of Straw Hemicelluloses

A broad variety of specific methods for the structure analysis of hemicelluloses have been established. The analysis of the

polysaccharide in question is always recommended because the complicated chemical structure, including branching, sequences of sugar units, the reduction groups in the end of chain, the glycosidic linkage, and the residual amount of naturally occurring impurities vary for a given type of polysaccharide and may significantly influence the properties and reactivity. So, the characterization tests are valuable as a guide in structural studies.

#### 4.3.2.1 Optical Spectroscopy

Optical spectroscopy can be used to determine the conformation of structural features of pure polysaccharide and to easily monitor structural changes during modification. Fourier transforms infrared (FTIR) spectroscopy yields “fingerprint” spectra usable as structural evidence. The most common way for FTIR measurements is the preparation of KBr pellets. To obtain well-resolved spectra, it is necessary to apply a ball mill to guarantee homogenous mixtures of KBr and the macromolecule. Usually, samples containing about 1–2% (w/w) polymer are prepared. Common “nonpolymer” signals observed by means of FTIR spectroscopy are adsorbed water at about 1630–1640  $\text{cm}^{-1}$  and  $\text{CO}_2$  at about 2340–2350  $\text{cm}^{-1}$ . A number of FTIR spectra obtained for the typical hemicelluloses from SCB and wheat straw hemicelluloses are shown in Fig. 4.7. The general assignment of straw hemicelluloses is given in Table 4.3.



**FIGURE 4.7** Fourier transforms infrared (FTIR) spectra of the sugarcane bagasse (SCB) hemicelluloses isolated using 10% KOH at 25 °C for 10 h from holocellulose (spectrum a) and wheat straw hemicelluloses (spectrum b) isolated using 10% KOH at 23 °C for 10 h from holocellulose.



**TABLE 4.3** General Assignment of FTIR Spectra of Hemicelluloses

Wave number (cm <sup>-1</sup> )	Assignment
3200 3570	OH stretch, intramolecular and intermolecular H bridge between the OH groups
2850 2980	CH <sub>2</sub> antisymmetric stretch and CH <sub>2</sub> symmetric stretch
1725 1730	C O stretch from acetyl or COOH groups
1640 1630	Adsorption of water
1455 1470	CH <sub>2</sub> symmetric ring stretch at pyran ring; OH in plane deformation
1416 1430	CH <sub>2</sub> scissors vibration
1374 1375	CH deformation
1335 1336	OH in plane deformation
1315 1317	CH <sub>2</sub> tip vibration
1277 1282	CH deformation
1200 1235	OH in plane deformation
1125 1170	C O C antisymmetric stretch (arabinosyl side chains)
1100 1110	C OH bending typical of xylans (ring antisymmetric stretch)
1040 1050	C O stretching in C O C glycosidic bonds
890 900	C1 group frequency or ring frequency (typical for $\beta$ anomers)

All the spectra are dominated by signals in the region 3600–2800 cm<sup>-1</sup> because of stretching vibrations of CH and OH and signals in the C–O stretching region (1200–950 cm<sup>-1</sup>) in Fig. 4.7. The sharp band at 1038 cm<sup>-1</sup> in the spectra is typical of xylans, indicating a dominant xylan of the isolated hemicelluloses. In the anomeric region (950–700 cm<sup>-1</sup>), a small sharp band at 890 cm<sup>-1</sup> is typical of  $\beta$ -anomers that indicated the presence of dominant  $\beta$ -glycosidic linkages between the sugar units. In the carbonyl stretching region, the bands between 1659 and 1573 cm<sup>-1</sup> are because of the adsorbed water [67]. The disappearance of signal at 1745 cm<sup>-1</sup> in all the spectra revealed that the alkaline extraction completely saponified the ester bonds, such as acetyl and uronic ester groups from straw hemicelluloses [89].

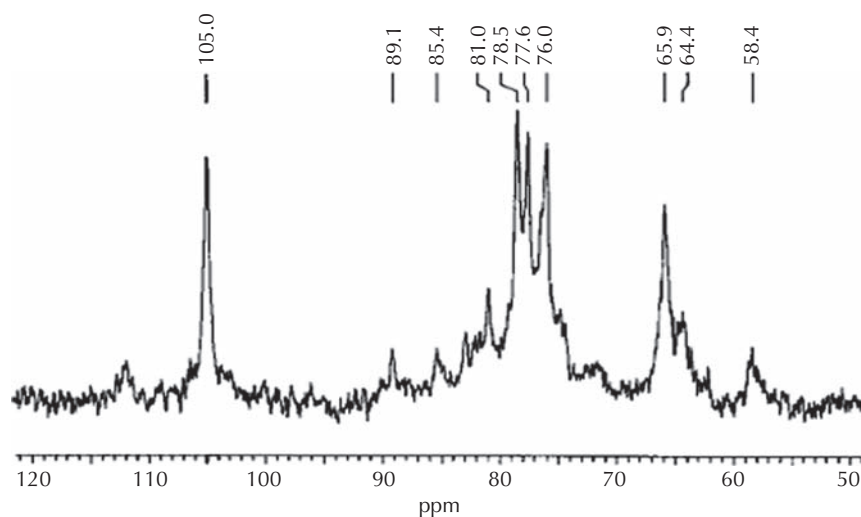
#### 4.3.2.2 Nuclear Magnetic Resonance Spectroscopy

Recent advances in the elucidation of the fine structure of hemicelluloses have been possible through developments in

appropriate analytical methods. In particular, nuclear magnetic resonance (NMR) has proven invaluable in studying the molecular structures of hemicellulose polymers and oligomers. For the most hemicelluloses, well-resolved <sup>1</sup>H-, <sup>13</sup>C-, and two-dimensional NMR spectra can be acquired from the solutions of the intact polymers in D<sub>2</sub>O, in DMSO-*d*<sub>6</sub>, and in other deuterated solvents. Sometimes, the application of solid-state NMR spectroscopy is necessary in the special case.

<sup>13</sup>C-NMR spectroscopy, a nondestructive probe of molecular structure, has become a method of choice for structure elucidation of native hemicelluloses [120, 122–124]. The measurements should be carried out at elevated temperature. Usually, the solvent applied has an influence on the chemical shifts of the signals. Measurements in D<sub>2</sub>O commonly lead to a downfield shift (higher ppm values) in the range of 1–2 ppm. Most of the resonances in the <sup>13</sup>C-NMR spectra of wheat straw hemicelluloses in Fig. 4.8 can be fully resolved and the assignments of the signals are well described. Five main signals at 105.0 (C-1), 76.0 (C-2), 77.6 (C-3), 78.5 (C-4), and 65.9 ppm (C-5) corresponding to (1→4)-linked  $\beta$ -D-Xylp residues, were obviously shown [125, 126]. The signals at 112.0, 89.1, 83.0, 81.0, and 64.4 ppm correspond to C-1, C-4, C-2, C-3, and C-5 of  $\alpha$ -L-arabinofuranosyl residues linked to  $\beta$ -D-xylans, respectively. Such groups of arabinose signals are typical of AX isolated from cereal straws [3]. The other signals observed at 85.4 and 58.4 ppm, respectively, are characteristic signals of C-4 and methoxyl group of a MeGlcA residue. All the data reported here are in good agreement with the structures of L-arabino-(4-O-methyl-D-glucurono)- $\beta$ -D-xylan already described in cereal straw or grass [126, 127]. <sup>13</sup>C-NMR spectroscopy allows for fast determination of the nature, configuration, and relative content of the monosaccharide residues constituting hemicelluloses as well as the type and amount of specific linkages, but it doesn't provide information about the residue sequence in the chain. However, the combination of methylation of hemicelluloses and <sup>13</sup>C NMR can show more information about the structure of wheat straw in Table 4.4. The main structural features of the L-arabino-(4-O-methyl-D-glucurono)-D-xylan from wheat straw were evaluated by means of methylation and <sup>13</sup>C-NMR spectroscopy [3]. Methylation of the hemicelluloses gave a product with an  $[\alpha]_D^{17}$  value of  $-87^\circ$  indicative of  $\beta$ -linkages, which was confirmed by the <sup>13</sup>C NMR spectrum ( $\delta$  104.8 for C-1). Reduction and hydrolysis of methylated fraction yielded the following methylated sugars: 2,3,5-tri-O-methyl-L-arabinose (6.8%), 2,3,4-tri-O-methyl-D-xylose (4.9%), 2,3-di-O-methyl-D-xylose (77.4%), 2-O-methyl-D-xylose (7.2%) and 3-O-methyl-D-xylose (3.7%). The formation of 2,3,5-tri-O-methyl-L-arabinose indicated the existence of 1 terminal arabinofuranosyl group per 13 xylose residues. The formation of a small proportion of 2,3,4-tri-O-methyl-D-xylose showed that





**FIGURE 4.8**  $^{13}\text{C}$  NMR spectra (in  $\text{D}_2\text{O}$ ) of the hemicellulosic preparation isolated with 2% $\text{H}_2\text{O}_2$  0.2%tetraacetylenediamine (TAED) at pH 11.8 for 12 h at 48 °C from 0.5 M KOH aqueous solution combining with ultrasonic irradiation for 15 min treated (35 °C, 2.5 h) wheat straw [75].

**TABLE 4.4** Methyl Derivatives from the Hydrolysate of Methylated Hemicellulosic Fraction and the Corresponding Chemical Shifts in  $^{13}\text{C}$  NMR Data (Adapted from [3])

Methyl derivative	Mole %	Chemical shift (ppm)					
		C-1	C-2	C-3	C-4	C-5	MeO
2,3,5 Me <sub>3</sub> Ara <sup>a</sup>	6.8	112.1	83.2	78.9	89.4	64.6	
2,3,4 Me <sub>3</sub> Xyl	4.9	104.8	75.9	81.4	78.9	66.1	
2,3 Me <sub>2</sub> Xyl	77.4	104.8	75.9	77.2	78.9	66.1	
2 Me Xyl	7.2	104.2	80.4	77.2	78.9	66.1	
3 Me Xyl	3.7	100.8	74.4	75.4	83.2	73.0	60.5

<sup>a</sup>2,3,5-Me<sub>3</sub>-Ara, 2,3,5-tri-O-methyl-L-arabinose, etc.

xylopyranosyl group also presented as a terminal unit, and there was one xylopyranosyl group among every 18 xylopyranosyl residues in the main chain. The existence of the backbone consisted of (1→4) linked β-D-xylose residues was indicated by the formation of a large proportion of 2,3-di-O-methyl-D-xylose. The side-chains were attached to positions 2 and 3 of the xylose residues, as indicated by the formation of 3 or 2-O-methyl-D-xylose. In addition, from the combination of  $^{13}\text{C}$ -NMR spectrum with sugar composition in hemicelluloses [3], the chemical shifts for D-glucuronic acid residues appeared at 100.8 (C-1), 75.4 (C-2, C-3), 83.2 (C-4), 74.4 (C-5) and 179.8 (C-6). The molar ratio of uronic acid: xylose in the hemicellulosic fraction 2 was 1.9:50. It can be concluded that for every 26 D-xylopyranosyl residues in the main chain, there was one uronic acid unit. Therefore, from the results of methylation and  $^{13}\text{C}$ -NMR spectroscopy, more

information about hemicelluloses structural feature could be obtained.

In addition to the detection of substructures, the general assignment of  $^{13}\text{C}$  NMR spectra discussed above is also useful for the elucidation of structural features of unknown polysaccharides. The assignment of the  $^{13}\text{C}$  NMR data may be carried out by two-dimensional NMR techniques and Distortionless Enhancement of Polarization Transfer using a 135 degree decoupler pulse (DEPT 135) NMR spectroscopy [128]. The DEPT NMR technique reveals whether a carbon carries a proton, and the degree of protonation (CH, CH<sub>2</sub>, or CH<sub>3</sub>). These analysis methods include two-dimensional NMR techniques, and DEPT 135 NMR spectroscopy have been used to analyze the structure of other polysaccharides, including dextran and cellulose, as well as the hemicelluloses before and after the modification.

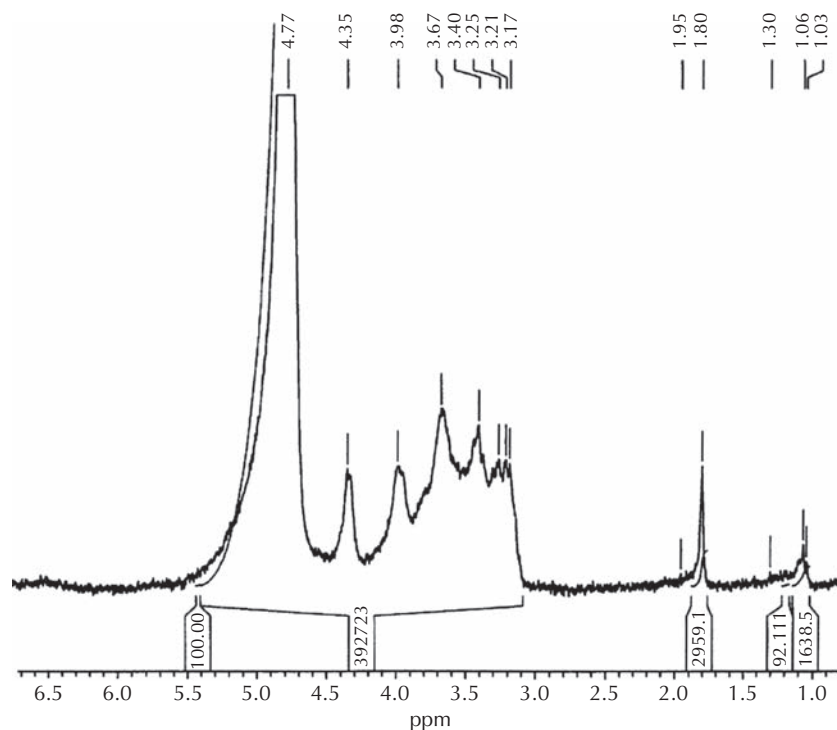


FIGURE 4.9  $^1\text{H}$  NMR spectrum of the degraded hemicelluloses dissolved in  $\text{D}_2\text{O}$  at room temperature [96].

$^1\text{H}$ -NMR spectroscopy is another useful tool to analyze the structure of polysaccharides. For example, in Fig. 4.9, the spectrum of wheat straw hemicelluloses, which were dissolved in  $\text{D}_2\text{O}$  at room temperature, showed that the chemical shifts of 3.2–4.4 ppm from the equatorial proton and other protons of anhydroxylose units of hemicelluloses. The methyl protons of fewer amounts of acetyl group and 4-*O*-methyl-D-glucuronic acid exhibit weak peak at 1.8 and 1.1 ppm, respectively. Anomeric protons of terminal  $\alpha$ -D-arabinofuranosyl residues give a shoulder at 5.2 ppm [129]. A strong signal at 4.7 ppm is originated from the residual solvent (hydrogen-deuterium-oxygen, HDO).  $^1\text{H}$ -NMR spectroscopy combined with monosaccharide and methylation analyses, and molecular mass estimation (FAB-MS, fast atom bombardment-mass spectrometry) have been proven to be an excellent approach for assigning unambiguous structures for oligosaccharides containing up to 14 residues [130, 131]. Because of the characteristic positions of the H-1 resonances in relation to the branching patterns in AX, the  $^1\text{H}$ -NMR data of oligomer fragments can be used in identifying specific structural domains present in the polymeric AX.

An interesting approach for the structure analysis of complex polysaccharides or mixtures of polysaccharides is the complete degradation (acid hydrolysis) and determination of the type of sugar and the concentration using the  $\alpha$ - and  $\beta$ -anomeric protons (H-1) as “probes.” This method is fast and reliable but gives no information about the linkage of the sugar components. Nevertheless, it can be

successfully applied in the analysis of lignocellulosic biomass (wood, pulp, agricultural residues, etc.) containing glucose, mannose, galactose, xylose, rhamnose, arabinose, and glucuronic acid [132]. Shin and Cho have used this technique to investigate the carbohydrate composition of woodmeal and pulps [133]. During acid hydrolysis, xylan monomer was dehydrated as furfural, and that furfural was further degraded or condensed in acidic reaction condition, therefore those reactions caused the lower xylose content in the compositional analysis of carbohydrate by anomeric hydrogen integration method [134]. Considering the furfural content, and conversion factor affected by other further degradation or condensation, the xylose content in woodmeal and pulps obtained by  $^1\text{H}$  NMR spectroscopy was similar to the values from gas chromatography (GC), liquid chromatography (LC), high performance liquid chromatography (HPLC), or high performance anion exchange chromatography (HPAEC) [133]. In this study, xylan conversion factor 0.66 was obtained based on compared NMR data from the prepared cellulose and xylan mixtures acid hydrolyzed with the same condition for woodmeal and pulps. With corrected xylan content calculation, NMR spectroscopic method gave rather closer carbohydrate composition compared with the other analytical methods. Although this approach has not been used to analyze straw hemicelluloses, obviously, it is proved to be an efficient method to determine the sugar content in hemicelluloses and will have bright future in the analysis of hemicelluloses.

### 4.3.2.3 Chromatography and Mass Spectrometry

The controlled depolymerization and the analysis of the sugars formed by means of chromatography, preferably gas liquid chromatography (GLC) and HPLC, are useful for structure characterization. For GLC studies, the sugars need to be converted to volatile derivatives. To avoid multiple peaks for the monosaccharides, modification of the C-1 aldehyde moiety is performed before the derivatization of the sugar –OH, –NH<sub>2</sub>, or –COOH groups. This is most frequently achieved by conversion of the aldehyde to an alditol with NaBH<sub>4</sub> in ammonia or in DMSO and alternatively by the formation of an oxime with hydroxylamine in Py or formation of a methyloxime [128]. Another simple procedure is the degradation of the polysaccharide with sulfuric acid or perchloric acid (70%, w/w) and separation of the sugars by means of HPLC (cationic exchange resin, e.g., BioRad Aminex HPX or Rezex ROA columns) using dilute sulfuric acid as eluent at elevated temperatures (65–80 °C). A summary of the retention time of different sugars commonly found in polysaccharides is given in Table 4.5. Usually, the application of a refractive index (RI) detector is sufficient. But the response factors of the sugars should be known or need to be determined. Alternatively, pulsed amperometric detection or ultraviolet (UV) detection can be used. If UV detection is applied, derivatisation of the sugars is recommended. The hydrolysis in combination with HPLC is very suitable for the determination and quantification of structural features and impurities. Recently, using HPAEC (high performance anion exchange chromatography with pulsed amperometric detector, HPAEC-PAD) to analyze the

sugar has become popular in the analysis field. HPAEC can detect both the monosaccharide and oligosaccharides. HPAEC has several advantages over other techniques, including high sensitivity, high efficiency, and the great ability of protecting sugar from converting into derivatives. Pulsed amperometric detection can also be used. The NaOH solution and acetate solution are usually used as the eluent. Many articles described methods using HPAEC to test the hemicelluloses polymers including straw hemicelluloses [116, 135–139].

Matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectroscopy is of increasing relevance for the precise analysis of complex polysaccharide molecules because of its sensitivity and applicability to analysis mixtures. It is a very helpful tool to analyze and quantify substructures in hemicelluloses [135, 136]. In the case of *O*-acetylated GX and glucomannans, the DS of acetylation can be determined by MALDI-TOF. The method can also be employed to evaluate the distribution of 4-*O*-methyl glucuronic acid residues along the polymer chain of hardwood and softwood xylans [140]. In addition, matrix-suppressed laser desorption and ionization mass spectrometry (MSLDI-MS) can also be used together with size-exclusion chromatography (SEC) distribution curve to obtain the average molar mass values and the polydispersity index for the xylan from spruce [141]. Up to now, this analytical technique has not been used in characterization of hemicelluloses from straw.

With the development of analytical techniques, more and more advanced analysis instruments have appeared recently. Because the main advantage of MS is that it not only provides information on the molecular mass of a compound of interest but also generates significant structural information. Analytical techniques have been coupled with MS, creating GC/MS, GLC/MS, HPLC/MS, and NMR-MS, which are probably the most useful for detection and identification of polysaccharide and modified polysaccharide.

**TABLE 4.5** Summary of Retention Times Found by HPLC for Different Sugars and Uronic Acids, with a Combination of a BioRad Aminex HPX and Rezex ROA Column Using 0.005 M Sulfuric Acid as Eluent at 65 °C (Adapted from [128])

Sugar	Retention time (min)
Arabinose	25.2 25.3
Fructose	23.5
Glucose	21.8 22.0
Glucuronic acid	19.5 19.7
Mannose	23.1 23.3
Rhamnose	24.6 24.7
Ribose	26.0 26.2
Xylose	23.3 23.5
Sodium acetate	34.8 34.9

### 4.3.3 Structure

Like most polysaccharides from plant, original hemicelluloses display a large diversity and polymolecularity. This corresponds to their presence in a variety of plant species and to their distribution in several types of tissues and cells. All straw hemicelluloses are characterized by a  $\beta$ -1,4-linked-D-xylopyranosyl main chain, which carries a variable number of neutral or uronic monosaccharide substituents [142]. These substituents such as arabinosyl, glucuronic acid, and acetyl substituents can be attached to the main chain at the two free OH groups of carbons C-2 and C-3 of the xylopyranose residue. The following are few typical examples of the structure of straw hemicelluloses from agricultural residues.

Recently, the wheat straw hemicelluloses have been widely investigated, and the structure of hemicelluloses from wheat straw has been explained by Sun et al. [3]. The hemicellulosic fraction isolated with 0.5 M NaOH at 37 °C for 2 h from wheat straw was confirmed to be a  $\beta$ -(1 $\rightarrow$ 4) xylan with side chains consisting of L-arabinofuranosyl and D-xylopyranosyl groups attached in position 3, and D-glucopyranosyluronic acid or 4-O-methyl-D-glucopyranosyluronic acid group attached at position 2. For every 13 D-xylopyranosyl residues in the main chain, there was one L-arabinofuranosyl group. For every 18 such D-xylopyranosyl residues, there was one D-xylopyranosyl group, and for every 26 such D-xylopyranosyl residues, there was one uronic acid unit (Fig. 4.10) [3]. In addition, to obtain a better insight into the structural characteristics, the hemicelluloses obtained from wheat straw have also been subfractionated into hemicelluloses A, B, and C by alkali (10% KOH 2% H<sub>3</sub>BO<sub>3</sub> at 20 °C for 16 h). Hemicellulose A, which was isolated from the supernatant by acidifying to pH 5.0 with acetic acid followed by centrifugation, is a more linear and less acidic fraction, whereas hemicelluloses B obtained from the mother liquor by precipitation with three volumes of 95% ethanol, then filtered and washed with 70% ethanol, is a more acidic and branched fraction. Hemicelluloses containing a high degree of side-chain substitution are more water soluble and bind less tightly to cellulose, whereas molecules with infrequent side chains are less water soluble and bind more tightly to cellulose [143].

In the review on hemicelluloses of cereals and grasses [144], nondendospermic xylans from CCs, fiber, and hulls displayed apparently unusual structural features, and were more complex than those normally isolated from wheat and other grass. Relatively, the structure of hemicelluloses obtained from maize stems was simpler than that of hemicelluloses from CCs. The hemicelluloses from maize stems are mainly L-arabino-(4-O-methyl-D-glucurono)-D-xylan. The  $\beta$ -(1 $\rightarrow$ 4)-D-xylopyranose backbone is

substituted by  $\alpha$ -L-arabinofuranose and  $\alpha$ -D-glucuronic acid as single-unit side chains [23]. However, hemicellulosic fractions A and B obtained from CC were different in composition and structural features. The major one (wis-AGX) is a low substituted arabino-(4-O-methylglucurono) xylan which is water insoluble. The second one (ws-AGX) is water soluble with a similar xylan backbone, containing in addition to the single arabinosyl and glucuronosyl branches also disaccharide side chains, composed of xylose and arabinose as well as minor amounts of galactose [145]. The corncob xylans showed the presence of a linear, water-insoluble polymer (wis-AGX) with ~95% of the backbone unsubstituted, and a water-soluble xylan (ws-AGX) having more than 15% of the backbone substituted [87]. Single  $\alpha$ -(1 $\rightarrow$ 2)-linked 4-O-methyl-D-glucopyranosyl uronic acid units and  $\alpha$ -(1 $\rightarrow$ 3)-linked L-arabinofuranose residues, as well as 2-O- $\beta$ -D-xylopyranosyl- $\alpha$ -L-arabinofuranose moieties constituted the side chains, which do not exist in rice straw hemicelluloses [24]. The uronic acid content was lower in the wis-AGX (~4%) than in ws-AGX (about 9%). A small proportion of the Xylp residues of the backbone are disubstituted by  $\alpha$ -L-Araf residues (Fig. 4.11). A peculiar structural feature of the ws-AGX is the presence of disaccharide side chains [ $\beta$ -D-Xylp-(1 $\rightarrow$ 2)- $\alpha$ -L-Araf-(1 $\rightarrow$ 3)]. This sugar moiety has been found usually esterified by FA at position 5 of the Araf unit and occur as a widespread component of grass cell walls [146]. Similar to acetyl groups, ester-linked FA is lost during the alkaline extraction of AGX. However, FA-containing ws-AGX preparations were isolated by ultrasonically assisted extraction of CC using hot water and very dilute alkali hydroxide solutions [70].

Another typical example is to describe the structure of SCB hemicelluloses. Compared with the structure of wheat straw hemicelluloses, the hemicelluloses from bagasse are much less studied in this respect. Up to now, only a few publications about the structure of hemicelluloses from SCB

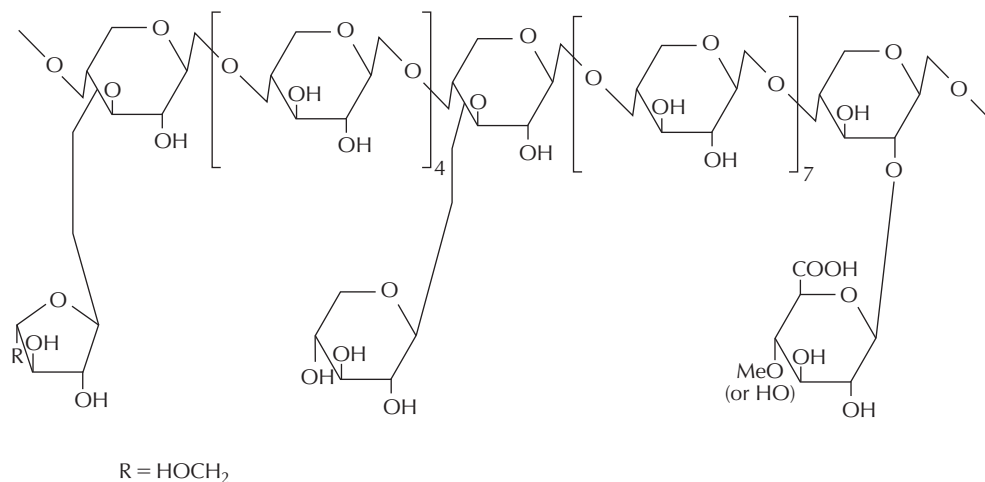


FIGURE 4.10 A structure of wheat straw hemicelluloses.

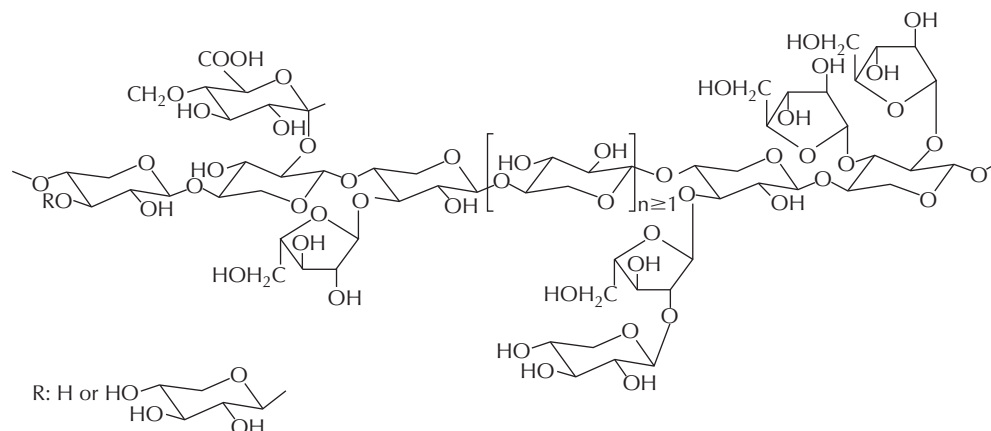


FIGURE 4.11 Primary structure of water soluble L-arabino 4-O-methyl-D-glucurono-D-xylan (ws AGX) in corn cob (CC) [1].

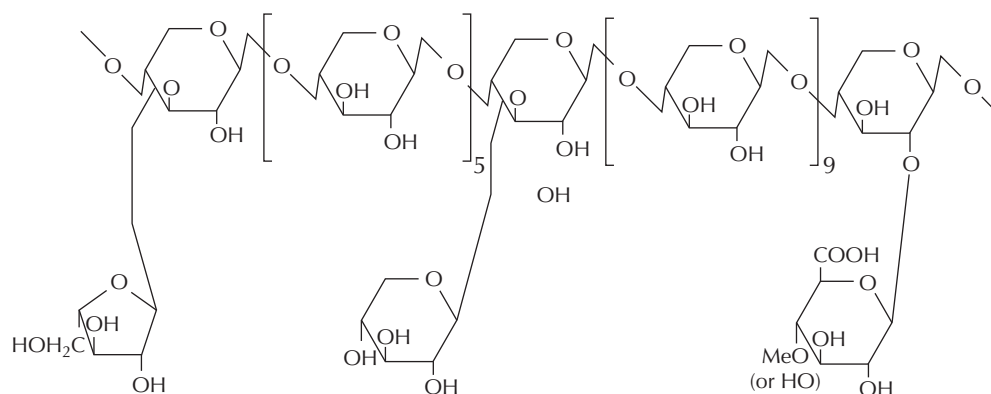


FIGURE 4.12 A structure of hemicelluloses from sugarcane bagasse (SCB).

have been reported [56, 83, 147]. These hemicellulosic polymers from SCB had a classical structure, with a backbone of  $\beta$ -(1 $\rightarrow$ 4)-linked xylosyl residues substituted with arabinose and MeGlcA at C-2 and/or C-3 of the main chain. The hemicelluloses from SCB are proved to be composed mainly of L-arabino-(4-O-methyl-D-glucurono) xylans, whose structure was brought forward by Sun et al. in Fig. 4.12, and which is similar to the structure of wheat straw hemicelluloses already described in previous article [143]. In addition, both alkali-soluble and acidic organic solvent-degraded hemicelluloses contained minor quantities of bound lignin and hydroxycinnamic acids, such as ferulic and *p*-coumaric acids [83]. The alkali-soluble hemicelluloses were more linear and acidic, and had a higher  $M_w$  and a higher thermal stability than those of the acidic organic solvent-soluble hemicellulosic fragments. Additionally, it was found that there exist small amount of *p*-coumaric acids and FA in the SCB cell wall. And *p*-coumaric acids are ester-linked to the cell wall components, mainly to lignin. Furthermore studies found that the site of feruloylation in bagasse hemicelluloses is exclusively the O-5 of arabinofuranosyl residue which is attached to the (1 $\rightarrow$ 4)- $\beta$ -linked xylan backbone at O-3 [148].

Rice straw is one of the main cereal straws, and it is produced in large quantities in many countries of the world every year. Less focus has been paid on the structure of hemicelluloses from rice straw, compared with those paid on the structure of wheat straw hemicelluloses. Sun et al. [23, 67] found a major difference between the water-soluble and alkaline peroxide-soluble hemicellulosic preparations being that the hemicelluloses in the former are mainly composed of  $\alpha$ -glucan, whereas the hemicelluloses in the latter comprise L-arabino-(4-O-methyl-D-glucurono)-D-xylan as the main constituent. The AGX had a backbone of  $\beta$ -(1 $\rightarrow$ 4)-linked xylosyl residues substituted with arabinose and MeGlcA at C-2 and/or C-3 of the main chain. Its structure is as follows in Fig. 4.13 [149]. In addition, the hemicelluloses obtained by alkaline peroxide treatment seemed more linear than those extracted by alkali in the absence of  $H_2O_2$ . There was no significant difference in the structural features of alkali-soluble hemicelluloses and alkaline peroxide-soluble hemicelluloses, which are mainly composed of L-arabino-(4-O-methyl-D-glucurono)-D-xylans. However, difference may occur in the fine structure of the polymers, that is, in the distribution of the branches along the xylan backbone.



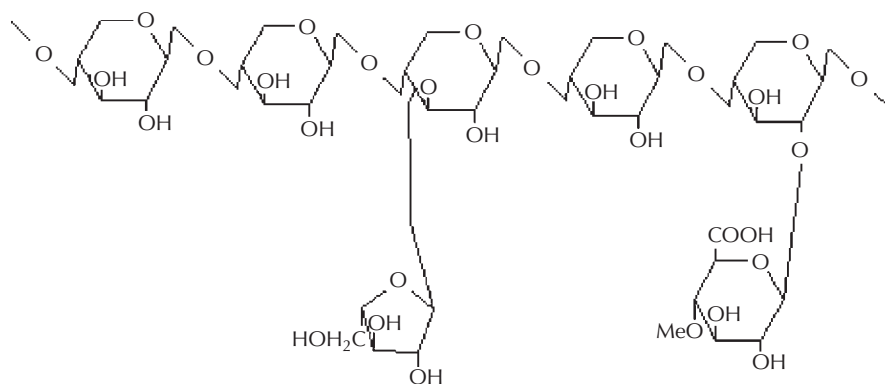


FIGURE 4.13 A typical structure of hemicelluloses from rice hemicelluloses.

Bamboo culms have received much attention as a potential biomass. Bamboo grasses are widely distributed in the world and have high xylan content. Although the bamboo, a renewable source for the production of xylose and xylooligosaccharides, is considered abundant, the structures of xylans from bamboo grasses have been poorly characterized. For example, the bamboo straw xylan from *Sasa senanensis* Rehd is a kind of AGX composed of D-xylopyranose, L-arabinofuranose, 4-O-methyl-D-glucopyranosyl uronic acid, and D-glucopyranosyl uronic acid [150]. The AGX is composed of the main chain of (1→4)-linked β-D-xylopyranosyl residues to which are directly attached α-L-arabinofuranosyl or α-(2-O-β-D-xylopyranosyl-L-arabinofuranosyl) stubs at the O-3 and 4-O-methyl-D-glucuronopyranosyl or D-glucuronopyranosyl stubs at the O-2 of the xylosyl residues of the main chain in Fig. 4.14. The corncob xylan also has the α-(2-O-β-D-xylopyranosyl-L-arabinofuranosyl) stubs at the O-3 position of the xylosyl main chain. Thus, the structure of this kind of bamboo grass xylan is similar to that of corncob xylan [151]. However, the bamboo grass xylan is somewhat different from those of rice straw [24] and bamboo (*Phyllostachys. bambusoides*)

[152, 153] xylans because the latter do not have the α-(2-O-β-D-xylopyranosyl-L-arabinofuranosyl) stubs (Fig. 4.15).

In brief, the hemicelluloses from reed were a kind of AGX, which is a backbone of β-(1→4)-linked xylosyl residues substituted with arabinose and MeGlcA at C-2 and C-3 of the main chain, respectively. For 16.3 such D-xylopyranosyl residues, there was one L-arabinofuranosyl group, and for 30.6 such D-xylopyranosyl residues, there was one uronic acid unit [149].

Timell [154, 155] reviewed the chemistry of hemicelluloses from angiosperms and gymnosperms, whereas Wilkie [144] described the structural characteristics of the different hemicelluloses from monocotyledonous species. However, their true primary structure is still unclear. For example, it's not known if the arabinosyl, uronic acid, or acetyl substituents are attached to the xylosyl backbone randomly or at regular repeating sequences. A significant portion of the xylose in cereal straw cell walls was found to be acetylated, mainly on C-2 but also on C-3 [154]. Bacon et al. [156] mentioned that cell walls of Gramineae plants account for 1–2% of the acetyl groups. Acetyl groups occur to the extent of 3–17% of wood hemicelluloses and are at

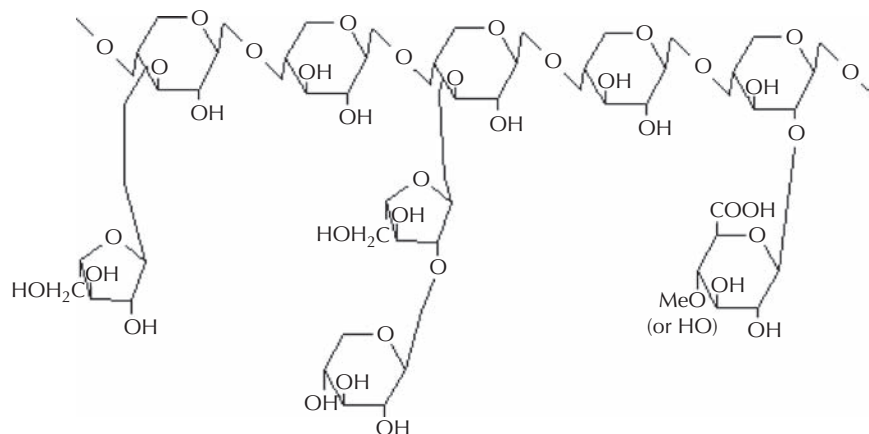
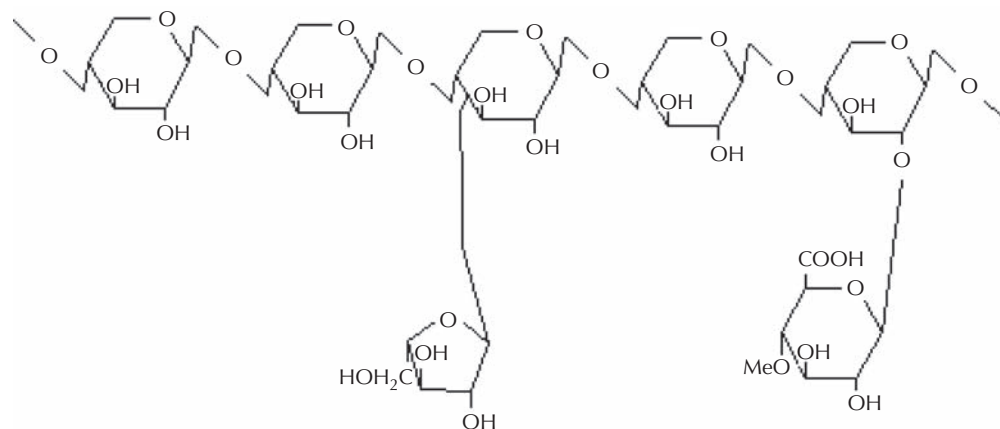


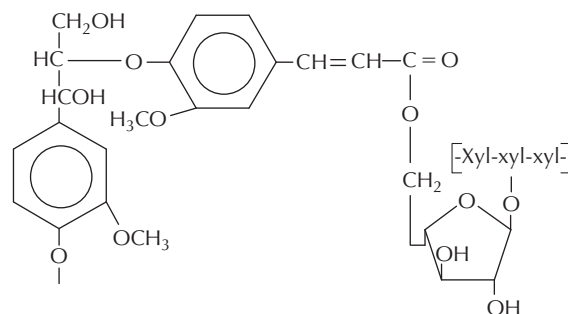
FIGURE 4.14 The typical structure of hemicelluloses from culms of bamboo (*Sasa senanensis* Rehd.).



**FIGURE 4.15** The typical structure of hemicelluloses from culms of bamboo (*Ph. bambusoides*).

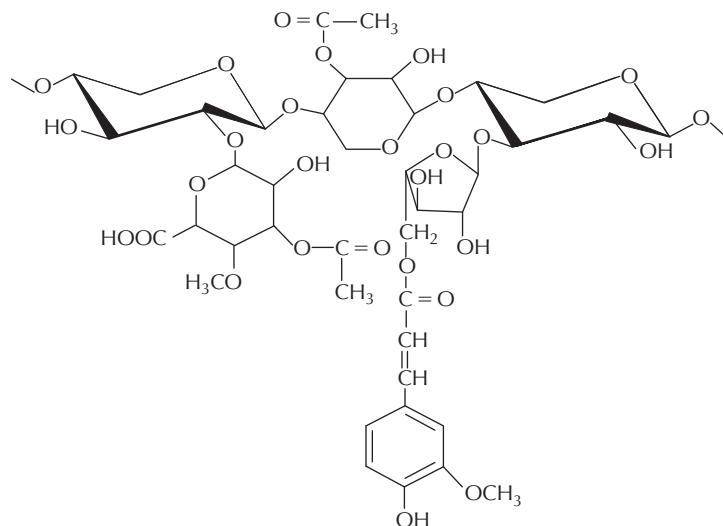
highest content in hardwoods. Dimethyl sulfoxide extraction of angiosperm woods yields hemicelluloses with 16.9% acetate groups corresponding on the average to 7.1 ester groups per 10 D-xylose units [157]. These acetylated hemicelluloses are soluble in water and in solvents such as dimethyl sulfoxide, formamide, and *N,N*-dimethylformamide (DMF). Furthermore, cell walls of cereal straws also contain 1–2% phenolic acids, which are esterified to hemicelluloses. *p*-Coumaryl and ferulyl groups are attached to the xylan through the arabinose residues at C-5 position (Fig. 4.16) [158]. It is estimated that one out of 121 pentose residues in ferulylated and one of 243 is *p*-coumarylated in barley straw [159].

FA is capable of forming both ester and ether linkages, and therefore, it may participate in cross-linking reactions of cell wall macromolecules, thus making the graminaceous matter less susceptible to digestion. It was reported that FA ether-linked to lignin formed a cross-link to hemicelluloses through an ester linkage (hemicelluloses-ester-FA-ether-



**FIGURE 4.17** Simple representative hemicelluloses ester FA ether lignin bridges.

lignin bridges). In these cases, FA ethers might form cross-links between lignin (at the  $\beta$ -position of the side chains) and hemicelluloses by simultaneous esterification of their carboxyl group to the C-5 position of arabinose substituents of AGX (Fig. 4.17) [160–171].



**FIGURE 4.16** A tentative 4-O-methylglucuronarabinoxylan with esterified acetic acid and ferulic acids [158].

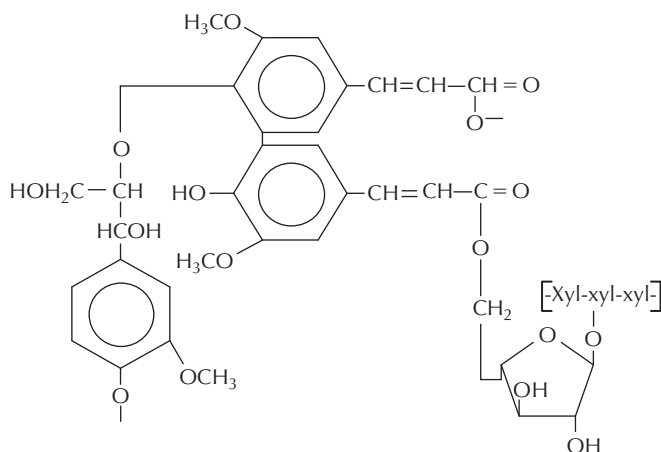


FIGURE 4.18 A simple representative hemicelluloses ester diFA ether lignin bridge.

Diferulic acids were also identified in the wheat straw cell walls by the cross-linking of AX to lignin (Fig. 4.18). There are between 9 and 10 FA ester-ether bridges for every 100 C<sub>6</sub> C<sub>3</sub> lignin monomers [172, 173], whereas most of the *p*-coumaric acids are mainly ester-linked to lignin at the  $\gamma$ -position of the side chains (Fig. 4.19). Only a few of them are esterified to hemicelluloses at arabinose substituents of AGX (Fig. 4.20). It should be noted that *p*-coumaric acid does not have a lignin/hemicelluloses cross-linking function [174, 175].

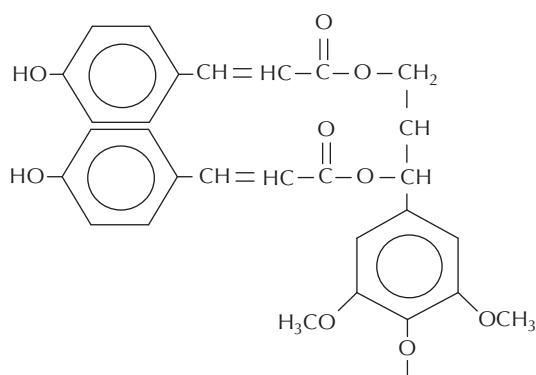


FIGURE 4.19 A simple representative *p* coumaric acid (PCA) ester lignin.

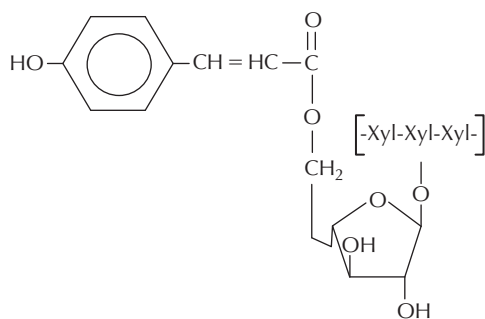
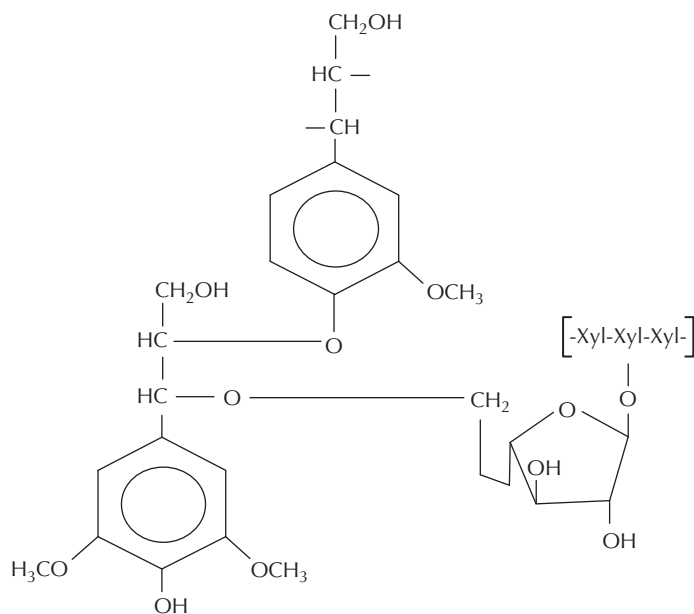


FIGURE 4.20 A simple representative PCA ester hemicelluloses.

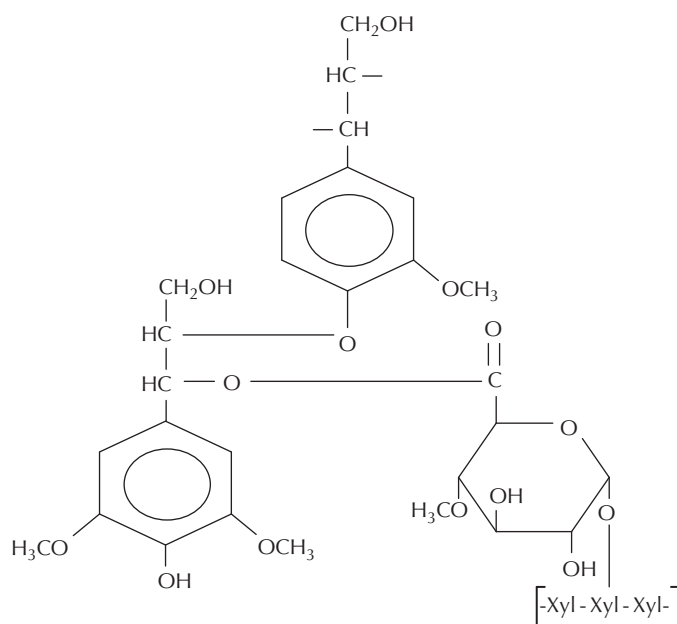
In addition to the linkages between hemicelluloses and hydroxycinnamic acids, the associations of xylans with other components of the cell wall also have commercial significance and have been studied. There is evidence that, during the assembly, the synthesis and deposition of xylan is intimately linked with cellulose [176]. Hemicelluloses have also been shown to be linked with secondary cell wall proteins. One example of specific secondary wall proteins located directly other than inferred from cDNA sequence, have been shown in Loblolly pine [177], hypocotyl of French bean [178], and in differentiating Zinnia cells [179, 180]. A particular protein (extensin), of which the uncommon amino acid hydroxyproline constitutes up to 20% of the amino acid content, is found in many dicots [181] as well as monocots [182]. This protein makes up 2–10% of the primary cell wall and most of the hydroxyproline residues are glycosylated by a tri- or tetraarabinoside [181]. Many of the serine residues are also glycosylated but with galactose residues. Hydroxyproline-rich glycoproteins containing arabinose residues have been isolated from monocotyledonous plants [183].

More importantly, the deposition of lignin in these walls involves generation of mesomeric phenoxy-radicals from the hydroxy-cinnamyl-alcohol precursors. These will rapidly form linkages with the hemicelluloses of the wall and these linkages may take place randomly [179]. Our previous studies found that the majority of lignins in cereal straw cell walls are directly linked to arabinose side chains of xylan by ether bonds [71, 184–186]. Chemical studies on these linkages between lignin and hemicelluloses, especially AX, have emphasized the important role of arabinose residues in forming these linkages. Chesson et al. [187] indicated the presence of a covalent association between arabinose side chains of xylan and phenolic substances including lignin in forage species. The presence of lignin arabinose linkages was also reported from spruce wood [188]. The majority of these benzyl ether linkages in wheat straw cell walls are etherified to the  $\alpha$ -position of the side

between the lignin and glucuronic acid residue of glucuronoxylan was determined to be 1.6 per molecular of lignin hemicellulose complex obtained from the beech wood. The occurrence of ester bond between lignin and glucuronic acid or 4-*O*-methylglucuronic acid in the cell walls of wheat straw was confirmed by the chemical analyses and was confirmed by <sup>13</sup>C-NMR spectroscopy studies [190–193]. In other words, in cereal straw cell walls,



**FIGURE 4.21** A simple representative benzyl ether linkage.



**FIGURE 4.22** A simple representative benzyl ester linkage.

hemicelluloses are usually ester-linked through the glucuronic acid side chains to lignin, and lignin polymers are attached to arabinosyl residues by aryl-ether linkages.

## 4.4 PHYSICOCHEMICAL PROPERTIES

Until now, the physicochemical properties of straw hemicelluloses have not been systematically investigated. Although most of the studies have been focused on the primary structure, there are a few attempts to establish relationships between the molecular structure and the physicochemical properties of hemicelluloses.

### 4.4.1 Solubility

It is a difficult task to prepare the solution of HX because of the poor solubility of most HX in aqueous and aprotic solvent systems, even for those originally isolated by water extraction. It makes  $M_w$  determination and characterization of solution properties become even more difficult. The solubility is known to be dependent on various factors described in the previous sections, such as the asymmetrical conformation, the DP, the type and DS by the various glycosyl side chains and their distribution pattern that is responsible for aggregation tendencies, the presence of acetyl groups, possible chemical linkages with lignin, and the cross-links through phenolic acids [11, 14]. For example, the DS of the xylan backbone by arabinofuranosyl residues determines the solubility of the xylan, in a considerable degree and also its ability to bind to cellulose. Xylan backbones containing a high degree of side-chain substitution are more water soluble and bind less tightly to cellulose, whereas molecules with infrequent side chains are less water soluble and bind more tightly to cellulose [194]. A recent study on alkali-extracted lignin-containing hemicelluloses indicated that the lignin component, present in bound and unbound forms, is the main contributor to aggregate formation [195]. The solubility of hemicelluloses is also affected by the patterns of intra- and intermolecular hydrogen bonds, which are natural or created during the drying process of the extracted polysaccharide as well as by storing [196]. To split off the hydrogen-bond, aqueous, aprotic, and complexing solvents, such as cuoxam, cadoxen, and FeTNa have been applied, and now they are also used in combination with physical treatments such as ultrasonication and autoclave heating. This treatments had been successfully applied to improve the solubility of XG from seed polysaccharide (the plant *Detarium senegalense* Gmelin) and Detarium gum [197, 198]. But the relationship between the structure and the solubility of straw hemicellulose is still barely investigated.

The extensive formation of hydrogen bonds in xylans, particularly low-branched xylans, during isolation and drying,

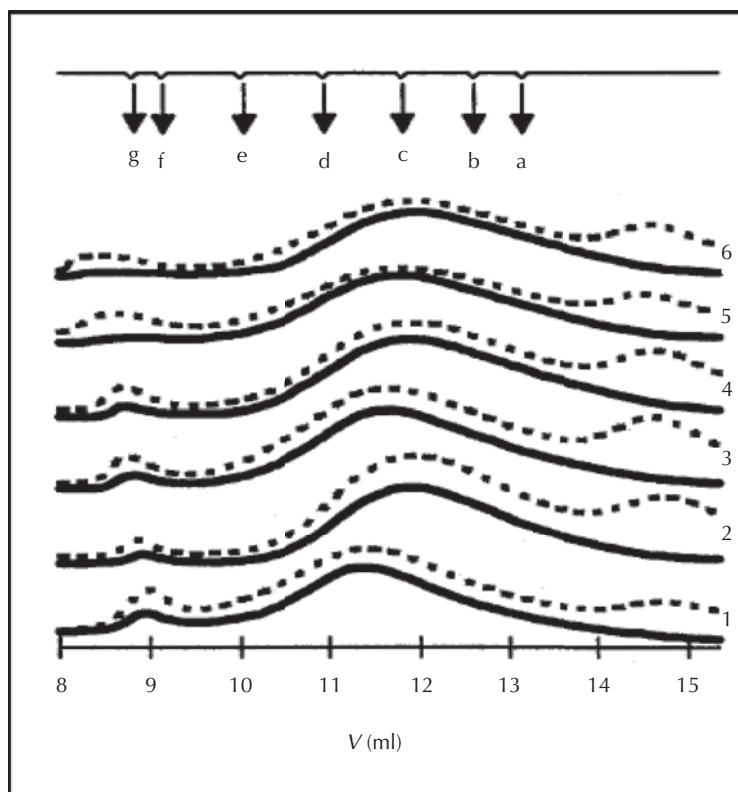
results in partial solubility or even insolubility of the polymers in water. Thus, the further conversion of hemicelluloses would be affected. Some treatment, like dispersing in hot distilled water, could facilitate the rapid conversion of xylan into a more uniform gel. The mixture of xylan and water would become a homogenous solution at 80–90 °C for 15–20 min [199, 200]. In addition, it was found that extensive and time-dependent molecular aggregation of hemicelluloses occurs in aqueous solution, and the effect is most apparent after heating the solution. And that there is a very tentative evidence that indicates the occurrence of conformational changes. All of the effects demonstrated are of potential importance in fractionation of hemicelluloses [200].

There are a few other solvents besides hot water that could dissolve hemicelluloses. DMSO was shown to be an efficient solvent for low-branched HX if 10% water was added and the solution was heated at 80 °C for 1 h before the measurement [196]. Sun et al. [201–203] investigated different solvents to dissolve hemicelluloses for the further homogenous chemical modification of hemicelluloses. Xylan-rich hemicelluloses from cereal straw can dissolve in *N,N*-dimethylacetamide/LiCl (DMA/LiCl) solvent. The mixture of 0.6 g hemicelluloses and 30 ml LiCl/DMA was stirred at 120 °C for approximately 2 h to form the homogenous system. Additionally, strongly polar aprotic solvents such as DMF were found to be able to prevent the aggregation of flexible hemicellulose chains, promoting the interactions between substrate and reagents. It needed about 2 h at 120 °C for hemicelluloses to completely dissolve in DMF/LiCl. Furthermore, ionic liquid is also shown to be an efficient solvent for hemicelluloses. Wheat straw hemicelluloses could dissolve completely in ionic liquids (ILs [1-butyl-3-methylimidazolium chloride]) at 90 °C for 1.5 h to form homogeneous system for further modification [204]. Crystal region in xylans disappeared after dissolving in ILs observed by polarizing microscope, which indicated the destruction of intra- and intermolecular hydrogen bonds by IL. The hydrogen bond network of xylans may also be reflected in the accessibility and reactivity of the functional groups in organic media. Therefore, the combination of the renewable raw material hemicelluloses with the recyclable ionic liquid is investigated to yield a contribution to environment protection and will be widely applied in the fields of chemical modification.

### 4.4.2 $M_w$ and $M_w$ Distribution

The  $M_w$  values reported for the various hemicelluloses shown considerable variation not only because of the above described structural difference in molecular groups but also the inconsistent estimation method even for the same sample [1, 4, 14]. For the water-extractable wheat AX (WAX), the  $M_w$  values (range from 65 000 to 66 000 g/mol) obtained by sedimentation [205, 206] are much lower than





**FIGURE 4.23** High performance gel permeation chromatography (HPGPC) records of corn cob (CC) xylan, ws AGX (1) before and after its sonication (2) in water at 200 W ultrasound power and 60 °C for 60 min, at 100 W and 60 °C in 1% NaOH for (3) 10 min and (4) 30 min, and in 5% NaOH for (5) 10 min and (6) 30 min RI (solid line) and UV detection (dotted line); markers indicate the elution volume of the pullulan standards (a) P10, (b) P20, (c) P50, (d) P100, (e) P200, (f) P400, and (g) P800; numbering denotes the  $M_w$  from 10 000 to 800 000 g N mol<sup>-1</sup>.

those obtained by gel filtration: 800 000 5 000 000 g/mol [20]. Comparable  $M_w$  values (150 000 and 162 000 g/mol) of the ws-AGX from CC were obtained by ultracentrifugation and viscometry coupled with static light scattering of fractions prepared by gel chromatography, respectively [145, 207]. Thus, extremely high  $M_w$  values were observed by light scattering techniques, gel chromatography, and coupled techniques such as size exclusion chromatography multiangle laser light scatter (SEC-MALLS). Chain aggregation was suggested to be partially responsible for the large variation in the estimates of  $M_w$  and also the presence of undissolved microgel particles [208]. The AGX with  $M_w$  value of 20 210 g mol<sup>-1</sup> for maize stems, 22 710 g mol<sup>-1</sup> for rye straw, 21 760 g mol<sup>-1</sup> for rice straw with the same treatment (the 1 M NaOH at 30 °C for 18 h) were obtained by gel filtration chromatography (GPC) [23]. Depending on the solvent quality, chain aggregation may be partially responsible for such a variation in the estimated weight of these polymers [19]. In spite of its higher DS, the corn bran HX form aggregates in solution and the  $M_w$  values could be estimated by static and dynamic light scattering (DLS) only after removal of the aggregates by filtration [209]. Therefore, the values determined are not representative for the whole xylan sample. Based on ultrasonication studies [210, 211], the high  $M_w$  components ( $M_w > 500\,000$  g/mol) of the CC

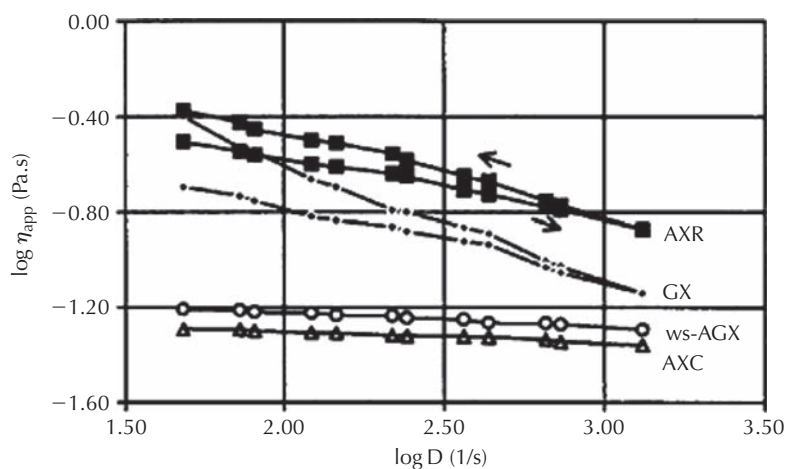
AGX and corn bran heteroxylan, were suggested to represent supramolecular structures (Fig. 4.23).

The elution profiles of wheat straw hemicelluloses indicate a very broad distribution of molecular size. The high ratio of weight-average  $M_w$  to number-average  $M_w$  ( $M_w/M_n$ ) reported for alkali-extractable wheat straw hemicelluloses (3.5–5.0) [53], organosolv soluble wheat straw hemicelluloses (5.0–5.8) [91], H<sub>2</sub>O<sub>2</sub>-extractable hemicelluloses from wheat straw with various organic solvents pretreatment (4.5–8.9) [76] strongly indicates (or states clearly) their inherent polydispersity.

The molecular properties of hemicelluloses are usually characterized by SEC using dextran, pullulan, or polystyrene standards for calibration [212–214] or by coupling of SEC with light scattering (LS) and viscometry [145, 209]. An advantage of the SEC technique is that the presence of protein and phenolic components or oxidative charges can be detected by simultaneous UV detection.

#### 4.4.3 Rheological Behavior

It is very important to study the rheological behavior of hemicelluloses for various applications. The rheological behavior of hemicelluloses is essentially affected by both



**FIGURE 4.24** Flow curves for 5% (w/v) aqueous dispersions of xylan polymers from various plant sources at 20 °C, GX (beechwood), ws AGX (corn cobs), AXR (rye bran), AXC (corn hulls) [4].

the primary structure (sugar composition, type of branches) and the fine structure comprising the distribution of segments or branches, shown in the previous sections. More information about rheological behavior of hemicelluloses extracted from wood and cereal flour and pulp have been mentioned in detail [215–225], but only a few articles reported the rheological behavior of hemicelluloses from cereal straw. Compared with the rheological behavior of beechwood GX, rye bran AX, and corn bran HX, the rheological behavior of AGX from CC was investigated and the results are shown in Fig. 4.24. The partially water-soluble GX isolated from beech wood exhibit shear-thinning behavior (typical for pseudoplastic polymers) and behave as plastic material at higher concentrations. This behavior mainly depends on the ionic state of the carboxyl groups of uronic acid moieties and on the content of the wis-GX fraction [217]. Strong thixotropy was observed also for the water-insoluble fraction of rye bran AX and CC AGX, whereas, the water-soluble fractions of AGX, AX, and corn bran HX were shear-thinning without thixotropy [145, 219, 220, 226]. The water-insoluble CC AGX formed a suspension of highly swollen particle which shows at approximately 4% distinct plastic behavior. The interactions between the insoluble but swollen particles of water-insoluble AGX seem to produce a stronger intrinsic structure than the soluble water-soluble AGX molecules. The deduced gel-like and liquid-like behaviors of both groups were confirmed by viscoelastic measurements.

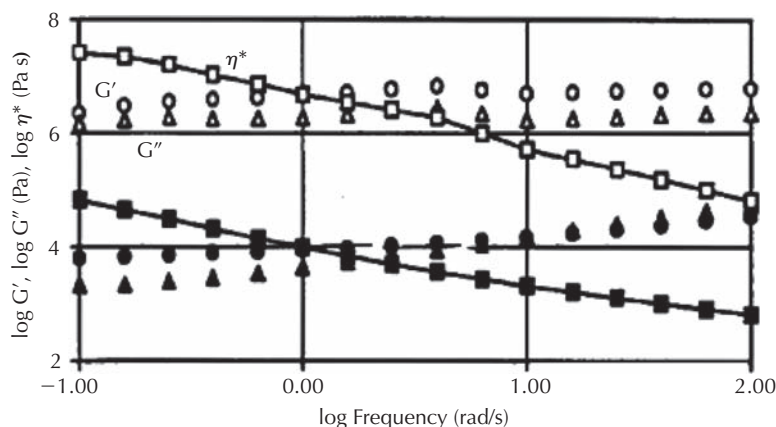
A strong structure-dependence was suggested from viscoelasticity studies [124]. The rheological behavior of AGX from CC was compared with GX from beechwood in Fig. 4.25. The mechanical spectra of GX dispersions show a weak-gel to gel-like character with increasing polymer concentration, and those of the water-soluble AGX and AX in the same concentration range are typical of liquid systems. However, the cereal flour AX and corn bran HX,

containing phenolic acids, are able to form gels only after oxidation [19, 220]. This was explained by the formation of cross-links between the FA units bound to both different xylan side chains [227, 228] and/or to proteins [229].

Moreover, the solution properties of two fractions of corn cob xylan (fraction A and B) differing in the uronic acid content were investigated by viscometry using DMSO and 4% NaOH as solvents [230]. The polymers presented good stability in alkali without depolymerization during the measuring time. All solutions showed Newtonian behavior in the concentration, and the shear-rate ranges were studied. Differences were found in the Huggins constant  $K'$ , which indicated flexible chains for A and rigid conformation for B, in accord with the low-branched and higher-branched backbone, respectively, of the fractions.

#### 4.4.4 Surface Tension

Polysaccharides are known to have significantly less surface activity in comparison with other biopolymers such as proteins. This characteristic is related to their hydrophilicity, and low chain flexibility. The behaviors of several AX preparations from cereal flour and cereal bran and beech wood MGX were reported [19, 231–234]. They were found to lower the surface tension ( $\sigma$ ) of water from 72.0 to 46.0 mN/m at the critical micelle concentration (c.m.c.) that is between 0.6 and 0.03% (w/v). Most of the xylans formed emulsions of the oil/water type with stability comparable with that of the commercial emulsifier Tween 20. The differences in the primary structure or viscosity of the xylans had no significant effect on the emulsifying activity. However, the presences of a low amount of proteins or lignin were supposed to contribute to these effects. The presence of small amounts of lignin and proteins acting as hydrophobic centers as well as the



**FIGURE 4.25** Mechanical spectra for 10% (w/v) aqueous dispersions of beechwood xylan, GX (empty symbols) and corn cob (CC) xylan, ws AGX (full symbols) at 30 °C,  $G'$ , Storage modulus;  $G''$ , loss modulus;  $\eta^*$ , complex dynamic viscosity [4].

film-forming effect of the polysaccharide were assumed to be responsible for such effects. In addition, they showed a significant protective effect against thermal disruption of foams that are formed by the surface active bovine serum albumin (BSA). Only the highly viscous beech wood xylan and the rye bran AX protein complex exhibited remarkable foaming activity, which was similar to that of gum Arabic. However, only few of the soluble xylan-type hemicelluloses from straw were reported to exhibit tensioactive properties [19].

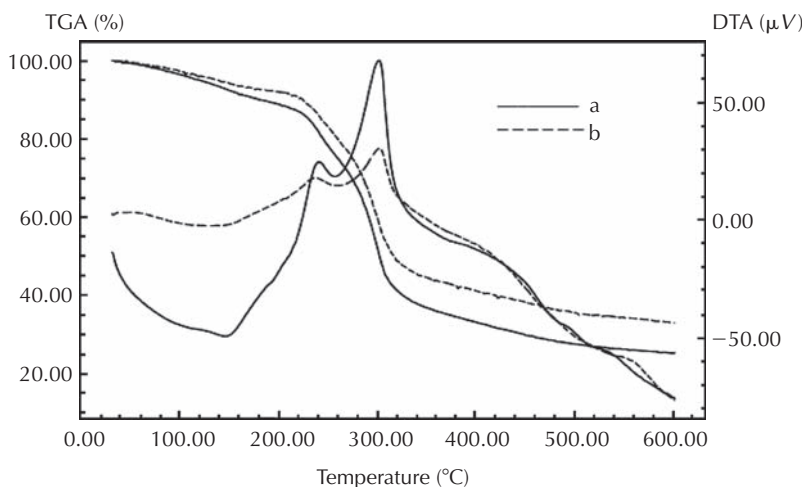
Film-forming properties of various pure xylans have been studied, but the results are not promising [125, 235]. In a recent study [236], xylans from CC, grass, and birch wood were added at levels up to 40% to wheat gluten to form biodegradable composite films. The results indicated that wheat/gluten composite films with different characteristics could be produced by varying the xylan type, composition, and process conditions without decreasing the film-forming quality and functionality such as the water-vapor transfer rate.

#### 4.4.5 Thermal Behavior

Xylan-type hemicelluloses represent thermally labile cell wall components of higher plants [237]. The thermal properties of xylans have been studied in the past only in relation to problems in wood and pulp processing [237, 238]. In some recent studies on xylans from straw, the thermal behavior has been characterized [23, 53, 56]. Similar to cellulose, xylans show no distinct thermal transitions. AGX preparations isolated under various alkaline extraction conditions from wheat straw [53] have shown a broad onset of thermal degradation near 200 °C. In this study, the thermal behavior was supposed to be affected by the presence of lignin. For the lignin-rich hemicellulose fraction, the differential scanning calorimetry (DSC) curves showed a prominent effect at 250–540 °C with three

maxima around 280, 435, and 500 °C corresponding to about 38, 20, and 13% of the total weight losses, respectively. The weight loss was interpreted as being due to decarboxylation in addition to dehydration and oxidation reactions of the carbohydrates and of less-condensed structures of the lignin component [239]. In the lignin-poor hemicellulose fraction, the peak maximum at 435 °C dominated.

The hemicellulose fraction, comprising about 75% of the hemicellulosic material, was isolated from delignified wheat straw. It consisted mainly of AGX and a minor amount of residual lignin [202]. The degradation of this fraction started at 220 °C and was completed at around 395 °C. In addition, for SCB hemicelluloses, four weight-loss stages can be seen in the curve of thermo-gravimetric/differential thermal analyzer (TGA/DTA) in Fig. 4.26, which is coincided with other hemicellulosic polymer studies [3]. The first weight loss occurred between 50 and 200 °C, which is attributed to the evaporation of water. The second, much faster step spread between 200 and 270 °C. It was followed by still faster step beginning at 270 °C and ending at ~340 °C, with a maximum weight loss rate at 322 °C, which relates to the major decomposition of hemicellulosic molecules. The fourth stage of weight loss ranging from 340 to 600 °C might be resulting from further breakage of the hemicelluloses. Moreover, it was also found that the hemicelluloses postextracted with a relatively higher concentration of alkali (10% KOH), had a slightly higher thermal stability than that of the hemicelluloses isolated firstly with a relatively lower concentration of alkali (8% KOH). All hemicellulosic samples have a residual weight between 25 and 35 wt% at 600 °C. In an inert atmosphere, the end-products of the decomposition of hemicelluloses are carbonaceous residues [240]. The inorganic compounds taken by the plants during growing, and the salts formed during the extraction and purification processes will contribute to the ash [241].



**FIGURE 4.26** Thermogram of two hemicellulosic fractions (spectrum a and spectrum b) extracted sequentially with 8% and 10% KOH at 25 °C for 15 h from the ultrasonic irradiated holocellulose [56].

#### 4.4.6 Biological Activity

Some interesting researches in searching of the biological activities of hemicelluloses have been carried out because of the biocompatibility, biodegradability, and high stability of these compounds. In the last decade, various biological activities of the hemicelluloses from birch wood [235], aspen wood [125], Shiitake mushrooms [242], and konjac glucomannan of herbaceous herb [243] have been revealed [244], while the biological activities of the others such as cereal hemicelluloses have not been exploited completely. For example, some of the xylan-rich hemicelluloses isolated from annual plant wastes such as bamboo leaves, corn stalks, wheat straw, etc. [245] have been reported to inhibit the growth rate of sarcoma-180 and other tumors, probably because of the indirect stimulation of the nonspecific immunological host defence. In addition, cereal xylans were found to contribute to the effects of dietary fiber on some biochemical and physiological processes in human and animal organisms [246–249]. Wheat bran AX has a suppressive effect on blood pressure [250] and the potential to bind hydrophobic mutagens in the diet [251]. Similar with rye bran AX, wheat bran AX shows an antinutritive effect [252] which was not observed for rice bran AX [253]. The ws-AGX from CCs [85, 211] exhibited significant immunostimulatory effects in the in-vitro rat thymocyte tests. Its activity was comparable with those of the commercial immunomodulator Zymosan, a fungal  $\beta$ -glucan. Ebringerova et al. [211] further found the disaccharide side chains on the backbone of CC ws-AGX might be important for the expression of the biological activity. Magnetite microparticles intended for oral intake and applied as magnetic markers for monitoring gastrointestinal motility were coated with xylan from CC [254]. The purpose of the polymer coating was to reduce dissolution of particles in the patient's stomach, ensuring passage into the colon.

Dissolution of magnetite was decreased significantly with the coating, as 28.5% of the uncoated particles were dissolved after 120 min in acidic media, whereas only 2.3% of the content was lost from the coated particles under the same conditions.

As progress in the pharmaceutical industry leads to increased demands on materials for specific applications, more specialized drugs and methods of drug delivery will be necessary to fulfill requirements. Plant cell walls are known to be a potential source of pharmacologically active polysaccharides. The promising attention will be focused on the investigation of biological activity of hemicelluloses, particularly for straw hemicelluloses.

#### 4.5 INTERACTIONS WITH CELLULOSE

The interactions of hemicelluloses with celluloses play an important role in many industrial processes, e.g., production of cellulose, papermaking, and bioethanol production. The effect of hemicelluloses on cellulose surface has been the focus of research efforts for a long time. Strong interactions present between hemicelluloses and cellulose as evidenced by the fact that the synthesis and deposition of hemicelluloses are intimately linked with cellulose during the assembly of the cell wall [176]. The strong interactions between hemicelluloses and cellulose also play an important role during the wood pulping process, where hemicelluloses dissolved from the wood can be retained to a considerable extent on the cellulose fibers [255–258]. The presence of hemicelluloses on cellulose fiber surfaces has been shown to enhance the strength properties of the fiber network [259, 260] by increasing the bonding ability of the fibers. The hemicelluloses retention phenomenon has been explained by cocrystallization of hemicelluloses segments with cellulose [256, 261] and the formation of



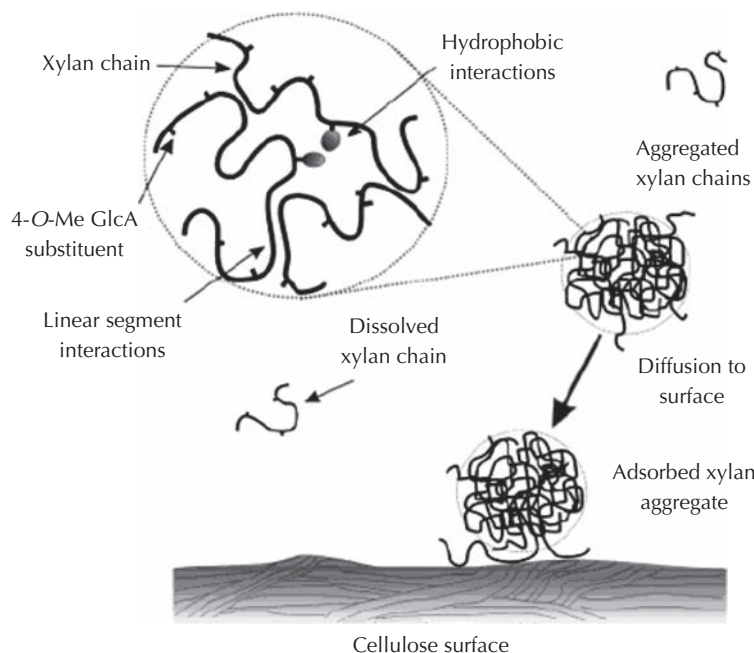
strong hemicellulose cellulose hydrogen bonds [262]. Also in processes dealing with the enzymatic conversion of cellulose from biomass to fermentable glucose to produce bioethanol, the interaction of xylans with cellulose needs to be reckoned [263].

#### 4.5.1 Interaction of Hemicelluloses with Celluloses in Production of Celluloses

In the production of cellulose-based materials, cellulose fibers usually have to be chemically functionalized to modify the interactions between the fibers or to adjust its compatibility with other materials. An attractive path is the use of hemicelluloses, e.g., xylans, mannans, and XG, which have a considerable potential in the developments of new high-performance materials with low environmental impact [264]. The happening of interactions between hemicelluloses and cellulose is possible because the linear xylan backbone allows a partial alignment and formation of hydrogen bonds to cellulose microfibrils. The xylans retained on the cellulose surfaces formed nano- and micro-sized particles by surface modification of cellulose fibers [265]. This is of practical importance that xylan-modified lignocellulosic fibers show improved wetting and liquid-spreading properties [266]. Thus, the study of xylan adsorption on cellulose may be a method of preparing new materials and composites.

Surface modification of cellulose fibers by assembly of birch GX from solution under pulping-like conditions has been previously reported [265]. When cotton linters were

exposed to a 5% water solution of xylan from birch wood at 110 °C for 2 h at pH value of 8, the substrates showed an increase in weight of approximately 6.5 %. The amount of adsorbed xylan could be controlled by variation of the autoclave treatment conditions. By using SEM and atomic force microscopy (AFM) analyses, it was found that, at higher yields, xylan forms regular, particle-like structures on the fiber surfaces. These particles were mostly of micrometer size or smaller and, thus, in a size range relevant for wetting and liquid spreading. It was likely that the assembly of xylan from solution onto the cellulose surfaces is not just crystallization but also an assembly process that is influenced by several factors such as changes in solubility and/or colloidal stability of xylan, or affinity between xylan and cellulose. Gatenholm and coauthors [267, 268] continued to investigate the mechanisms of xylan assembly in solution and the formation of xylan surface structures on cellulose. It was found an increase in both size and amount of xylan aggregates happens with increased treatment time. The increased aggregation can be explained by the decreased amounts of solubilizing 4-*O*-methyl glucuronic acid substituents in the autoclaved xylans. The retention of xylans on cellulose surfaces under conditions similar to those in wood pulping is likely to take place through an adsorption process involving diffusion to, and subsequent interaction with, cellulose surfaces of preformed xylan aggregate structures. The mechanism for xylan retention on celluloses was proposed by Gatenholm and his coauthors [267]. Diffusion to and adsorption onto cellulose surfaces can occur both in the case of single molecules and in aggregates (Fig. 4.27). This showed that the aggregates



**FIGURE 4.27** Schematic illustration showing the mechanisms for xylan aggregation in solution and interaction with cellulose surfaces [267].



constitute the major part of the adsorbed xylan. The xylan molecules can associate through interactions between the unsubstituted, linear regions of the chains. Furthermore, the xylylans contain small amounts of aromatic substituent, that is, covalently bound lignin residues, which can promote association by hydrophobic interaction. Both these factors favor aggregation. However, the presence of negatively charged 4-*O*-Me-GlcA substituents on the xylan chains increases repulsion between xylan molecules and favors dissolution. Autoclave treatment at high temperature and pH leads to decreases in the amounts of 4-*O*-Me-GlcA groups on the xylan backbone, thereby increasing the driving force for xylan aggregation. The larger and more numerous aggregates in the solution, probably also having lower solubility, ultimately lead to increased adsorption of xylan onto the cellulose surfaces that present.

Although so many researches are focused on xylan absorption, little is known about the adsorption of structurally different xylylans to cellulose [265, 267, 269 273]. Therefore, the adsorption of various xylylans (WAX, oat spelt [OS] xylan, hardwood xylan [Euc xylan]) to bacterial cellulose (BC) was studied [273]. The relationship between structural characteristics of xylylans and the adsorption onto BC was studied by determining the adsorption isotherms of the four purified xylylans: WAX, OS xylan, Euc xylan with *O*-acetyl groups, and Euc xylan without *O*-acetyl. The results are shown in Fig. 4.28. The four adsorption isotherms all showed a different course, which could be divided in two parts: a first steep increase of the adsorption isotherm (Fig. 4.28), and an increase in the adsorption isotherm after the first steep part. Only for WAX, a maximum value of adsorption was reached. In general, unsubstituted linear xylan parts help adsorption to BC. Xylan affinity for BC was also related to xylan size. The presence of arabinosyl and *O*-acetyl substituents to xylan decreased the adsorption of xylan to BC considerably. Removing substituents resulted in higher amounts

of adsorbed material. Most likely, increasing the number of unsubstituted xylyl residues induced the formation of xylan xylan interactions, which contributed to adsorption to BC.

Up to now, only a few publications have mentioned the adsorption of agriculture straw xylan to cellulose, because the investigations have not been focused on straw as sustainable materials until recently the shortage of fuel in this world makes sustainable materials like straw particularly concerned. Esker et al. [274] comparably investigated the self-assembly behavior of the different structure of hemicellulose-related heteropolysaccharides from peanut hulls and rice husks, yellow poplar wood, and rye straw and corresponding hemicellulosic derivatives such as cationic and anionic xylan on generated cellulose film. It was found that the amount of adsorbed xylan (heteropolysaccharide) is promoted by cationic charge density as well as the enhanced amphiphilic character with strong hydrophobic substituents. In other words, both ionic and hydrophobic interactions are important to xylan adsorption on cellulose surfaces. The effect of barley husk AX adsorption on the properties of cellulose fibers including cotton linters, regenerated cellulose fibers (Lyocell), and softwood kraft pulp was reported by Kohnke et al. [275]. Temperature and initial concentration of barley husk GAX are important parameters in controlling the level of adsorption on cellulose fibers, but the adsorption appears to be mainly determined by the xylan molecular structure, which agree with the comments proposed by Kabel [273]. Xylylans with a low DS have an enhanced capacity to interact with each other and with cellulose; this feature explains the observed propensity of low-substituted xylan to form agglomerated structures and adsorb on cellulose surfaces. This structure property relationship is, thus, of great importance when controlling the level of xylan adsorption, as well as the organization of the assembled layer.

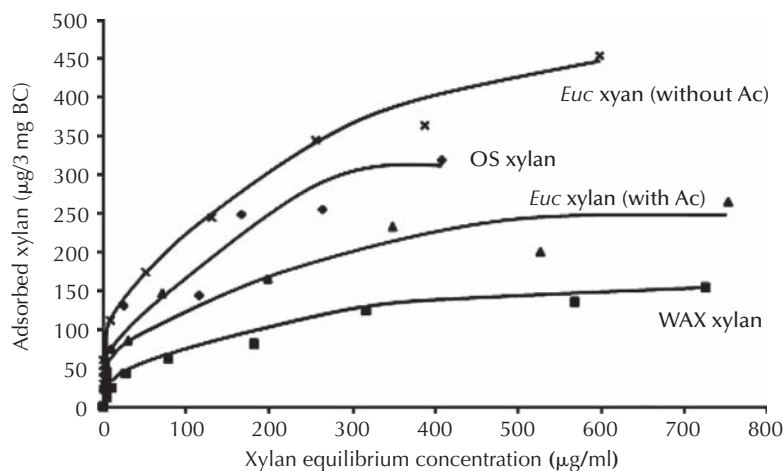


FIGURE 4.28 Adsorption isotherms of four structurally different xylylans adsorbed to bacterial cellulose (BC) [273].

### 4.5.2 Interaction of Hemicelluloses with Celluloses in Papermaking Process

Hannuksela and coauthors simply mentioned the sorption mechanism of hemicelluloses with fiber surface for papermaking process in a previously published article [276]. In general, the sorption of hemicelluloses onto fiber surface is usually simply described as a process involving four steps [277]. The first involves the diffusion or transport of hemicelluloses to the fiber surface. This process is governed by the collision frequency induced either by Brownian motion or turbulent transport. Attachment or sorption of hemicelluloses to fiber surface, in the second step, is a direct consequence of collision if the polymer sorbent attraction is higher than the polymer solvent attraction. As a third step, the hemicelluloses will undergo certain rearrangements or reconfigurations after the attachment at the fiber surface, during which hemicelluloses are also believed to diffuse into the fiber. The last step involves the reverse of steps 1–3 or the detachment of hemicelluloses from the fiber surface. The extent of desorption is, however, believed to be quite limited. Therefore, the sorption of hemicelluloses with fiber surface will affect the property of fibers.

Sorption of hemicelluloses not only affects the mechanical properties of pulp with main cellulose but also affects the beat ability of pulp. As a result of their hydrophilic properties, addition of hemicelluloses favors interfiber bonding, which causes higher swelling of the cell walls in water, thus imparting fiber flexibility, and assures better fiber conformation and more interfiber bonding during sheet formation. Consequently, the strength properties of paper, correlated with interfiber bonding, viz., tensile index, folding endurance, etc., are improved. In addition, hemicelluloses may contribute to more hydrogen bonding per unit area than cellulose surfaces do [278]. Thus, their addition to the pulp, resulting in concentrated absorption on the external fiber surfaces, explains their effect as additives in fiber bonding [279]. The content of hemicelluloses favors interfiber bonding, and therefore, the breaking length, burst, and folding endurance of the paper sheets are improved. The effective components are not all hemicelluloses, neither of the gross of hexosan. Moreover, the location and charge of hemicelluloses have a considerable impact on the formation of interfiber bonds. The charge of hemicelluloses is important for fibers' bonding. Chemical sorption of anionic xylan increased the charge of the fibers and, accordingly, the water retention value of the fibers. Thus, fibers' charge appears to affect the drying restraint of the fiber network [260]. During the last decade, more sorption experiments were performed with rather pure and well-characterized mannans, xylans, or hemicelluloses from waste liquid. In 1998, Hu [280] investigated the hemicelluloses obtained from the waste liquor of magnesium hydrogen sulfite of reed as surface-sizing agents, to improve

the strength of base paper of corrugated board. Laine and Stenius [281] found an increased swelling of hardwood and softwood kraft pulps with increased fiber charge, and their results also indicated the importance of hemicelluloses with this regard. According to the authors, swelling leads to higher sheet density and a higher relative bonding area, which might be partly because of anionic uronic acids groups of hemicelluloses. Suurnakki et al. [282] investigated the effect of galactoglucomannan on softwood kraft pulps by applying a sequence of sorption experiments and enzymatic peeling tests. The natural mannan content mainly affected the density of the fiber network, while sorption experiments increased the water retention and tensile strength. Hannuksela et al. [283] reported similar effects for the addition of mannans from thermomechanical pulp and guar gum, partly after enzymatic modification. Various XG and galactomannans to eucalypt kraft pulp were investigated by Lima et al. [284]. XG had the best effect on strength properties. The maximum burst strength was found after 1% (w/w based on pulp) xyloglucan addition, while a maximum for both tensile and tear values was found in the case of 5% xyloglucan addition. Schonberg et al. [260] observed an increased tensile strength after glucuronoxylan addition to pulp. The efficiency of dissolved hardwood xylan as a specific surface-modifying agent in the softwood kraft cook was investigated [285]. The gain in strength was correlated to the molecular mass of the xylan adsorbed and to the increased surface charge of the fibers. Hemicelluloses obtained from waste liquid of bagasse pulp by soda method was used as surface-sizing agent in recycling pulp [286]. This method is to spray the solution of hemicelluloses on the fibers sheets in drying process. In this process, the macromolecular of hemicelluloses is rapidly coated on fibers on the surface of sheets to form bonds after drying. Compared with commercial starch used as surface-sizing agent, in some cases, hemicelluloses is preferred to improve the property of sheets, for example, burst strength and compressive strength under plane.

Straw, as a source for xylan type hemicelluloses which could be used for quality improvement of pulps, is a cheap agricultural residue. A few articles reported agricultural straw xylan adsorption. Naterova et al. [287] added 2% CC AX to packaging papers and improved the modulus of rupture by 172%. Saake et al. [288] compared the effect of OS AX and birch 4-*O*-methylglucuronoxylan on various pulps. In the absence of other paper additives, the AX was superior to the 4-*O*-methylglucuronoxylan as manifested in higher strength improvements. However, together with cationic paper additives, the xylans had a pronounced synergistic effect, and the AX showed only a small advantage with this regard. The addition of AGX from SCB into different pulp with main cellulose in old corrugated container (OCC) pulp, thermomechanical pulp (TMP), and bleached kraft pulp (BKP) also have been considered by Ren et al. [289–291]. It was found that the

addition of SCB AGX into pulp with main fibers could improve the strength properties of hand sheets, which was because of the hydrogen bonds formed between hemicelluloses and celluloses during formation of sheet. Ramirez et al. [292] investigated sorption of CC and OS xylan onto bleached and unbleached softwood kraft pulp. The xylan sorption rate was strongly improved by the prolonged beating of pulp and highest sorption rates were determined for NaOH-OS xylan. This indicated that the sorption was mostly affected by the substitution pattern. In the case of NaOH-OS xylan, the higher arabinose content played an important role. The strength properties of handsheets were improved most notably by addition of the alkaline extracted xylans. Thus, high molar masses of xylan seemed to be beneficial for strength improvement. Highest sorption rates were observed for the NaOH-OS (12.32%) and lowest for the SE-CC xylan (6.614%). In general, the alkaline extracted xylans have a better performance, most likely as a result of their higher molar masses. The addition of xylan might be more suitable for products which require only low beating degrees and high fiber stiffness, e.g., in tissue production. Barley husk GAX showed potential as a dry strengthening agent [275]. The addition of xylan would decrease the need for mechanical refining of pulp in the paper-making process and, thus, would reduce the consumption of electrical power. The effect of GAX adsorption on paper strength was also investigated. A GAX-modified kraft pulp showed an evident increase in tensile strength, which might be because of a retained fiber fiber bonding ability for xylan-coated fibrils after drying and rewetting, which are consistent with the results brought forward by Paananen [293].

#### 4.5.3 Effect of Interaction of Hemicelluloses with Celluloses on the Bioethanol Production

Recently, lignocellulosic materials such as agricultural residues have been recognized as potential sustainable sources of mixed sugars for fermentation to bioethanol in the context of increasing prices of petrochemicals and concern for the environment. To obtain a high overall ethanol yield and to achieve an economically feasible production process, the removal of lignin and hemicelluloses is necessary to improve the accessibility of cellulosic materials to hydrolytic enzymes and avoid the degradation products. As well known, hemicelluloses are the most complex components in the cell wall of woods, straws, and grasses. They form hydrogen bonds with cellulose, covalent bonds with lignins and ester linkages with acetyl units and hydroxycinnamic acids [294]. In addition, structurally, hemicelluloses and pectins are important components of the cell walls, providing the matrix in which the crystalline

cellulose elementary fibrils are embedded. It is in this role that hemicelluloses becomes a barrier to efficient cellulose function and thus must be removed for efficient overall cell wall saccharification [295]. Therefore, the interactions of hemicelluloses with celluloses need to be taken into account significantly before removal of hemicelluloses and lignin for bioethanol production.

Lignocellulosic biomass is generally resistant to enzymatic degradation in its native state because of the complex linkage among cellulose, hemicelluloses, and lignin. So developing a highly effective eco-friendly method for the removal of hemicelluloses and lignin from lignocellulosic materials before bioethanol production from cellulose is necessary. Various pretreatment options such as acid hydrolysis, alkali, steam explosion, and wet oxidation are available for pretreating lignocellulosic biomass, all of these processes enhanced the enzymatic digestibility of biomass to a certain extent, but none of these methods was suitable for commercial application. Recently, steam explosion has been widely used to pretreat wheat straw [106], barley straw [296], and rice straw [297]. The steam will dash out from inside of the lignocellulose to damage lignin sheath that embeds celluloses and hemicelluloses, and damage hydrogen bonds between hemicelluloses and celluloses and, accordingly, increasing the contact of cellulose with enzyme. Furthermore, chemical pretreatment is to use the chemical to break the linkage among cellulose, hemicelluloses, and lignin. For example, alkali treatment of lignocellulosic substances disrupts the cell wall by dissolving hemicelluloses and lignin in aqueous media, hydrolyzing uronic and acetic acid esters, swelling cellulose and decreasing the crystallinity of cellulose [298], and increasing the biodegradability of the cell wall as a result of cleavage of the bonds between lignin and cellulose [299]. The effective pretreatment by alkali has been reported to be used to wheat straw [300, 301] and CC [302]. Dilute acid treatments can be used to hydrolyze hemicelluloses to sugar with high yields, change the structure of the lignin, and increase the cellulosic surface area. Usage of mild acid pressurized hot water for pretreatment of wheat straw [303, 304] and SCB [305], and corn stovers [306, 307] for bioethanol production was investigated. In addition, lime pretreatment has been studied on various biomass substrates such as wheat straw [308, 309], corn stover, wood, and municipal wasters [310, 311]. Alkaline peroxide pretreated wheat straw has also been reported [312]. Alkaline peroxide is an effective agent for liberation of both hemicelluloses and lignin from cellulose in cereal straw and grass by oxidation of lignin structures, cleavage of some interunit bonds and, eventually, the dissolution of lignin and hemicelluloses. Alkaline/oxidative has been recognized as a powerful oxidizing agent. To decrease the alkali and water consumptions, Cheng et al. investigated mild alkaline/oxidative pretreatment for SCB during ethanol production [313]. A summary of various pretreatment options is given

**TABLE 4.6** Methods for Pretreatment of Lignocellulosic Biomass (Adapted from [314])

Method	Example
Thermomechanical	Grinding, milling, shearing, extruder
Autohydrolysis	Steam pressure, steam explosion, and supercritical carbon dioxide explosion
Acid treatment	Dilute acid and concentrated acid
Alkali treatment	NaOH, ammonia, and alkaline H <sub>2</sub> O <sub>2</sub>
Organic solvents treatment	Methanol, ethanol, butanol, and phenol

in Table 4.6. Each pretreatment method offers distinct advantages and disadvantages. In general, the aim of those pretreatment methods of lignocellulosic biomass is to liberate hemicelluloses and lignin from cellulose in the cell wall for producing bioethanol.

#### 4.5.4 The Assembly Characteristics of Hemicelluloses on Cellulose

The assembly characteristics of hemicelluloses, including xylans, and their interactions with cellulose have been intensively studied. A combination of different analytical techniques is required. Methods using spectral fitting of CP/MAS <sup>13</sup>C-NMR spectra [315], DLS, as well as AFM or field emission scanning electron microscopy (FE-SEM), and Quartz crystal microbalance with dissipation monitoring (QCMD) [267, 293, 316] were developed to diagnose and monitor interactions between cellulose and xylan. FE-SEM and AFM allow for observation of the surface fibril aggregates, and additionally AFM can be used to investigate the forces between cellulose beads in aqueous solutions of simple electrolyte and hemicelluloses. QCMD studies the adsorption kinetics and characteristics of adsorbed hemicelluloses on cellulose. DLS are often used to study the solution and aggregation behavior of hemicelluloses as they allow the determination of dimensions and architectures of macromolecules in solution [267]. The average fibril and fibril aggregate (macrofibrils) width can be estimated by CP/MAS <sup>13</sup>C-NMR. This is done by spectral fitting of the experimental NMR spectra. The sizes of cellulose fibril and fibril aggregates estimated by CP/MAS <sup>13</sup>C-NMR have been shown to be consistent with those of similar samples reported in the literature [317, 318].

In conclusion, the interaction of hemicelluloses with celluloses is still popular subjects because it plays important roles in various applications such as production of celluloses, process of pulping, and production of bioethanol, and so on. However, the interacting mechanism of

hemicelluloses with cellulose needs to be fully understood. A great advantage of using hemicelluloses for cellulose fiber modification is that the two polysaccharides have natural affinity for each other. Preisolated xylans from agricultural residues have not yet been used commercially in large-scale cellulose fiber modification despite their obvious potential as renewable polymers. The major reason for this, probably, is the high process costs associated with large quantity xylan isolation. However, residues from agriculture represent a low-cost source of xylan-containing material that could be turned into an industrial opportunity if it is processed into value-added products, where CC, barley husks, wheat and rice straw, as well as SCB have shown to have great potential.

## 4.6 MODIFICATION OF HEMICELLULOSES AND ITS APPLICATION

In current trend for a complex and more effective utilization of biomass, increasing attention has been paid during the last few years to the exploitation of hemicelluloses as biopolymer resources. Hemicelluloses are available in very large amounts in organic wastes from renewable forest, and agricultural residues such as wood meal and shavings, stems, stalks, hulls, cobs, husks, etc., and they can be relatively easily extracted from biomass. So conversion of so much amount hemicelluloses to useful products may provide a fundamental solution to shortage of the natural energy sources and replacement of petroleum-based products. In the past, the research activities in the fields of hemicelluloses were aimed mainly to utilize the plant biomass by conversion into sugars, chemicals, fuels and as sources of heat energy. However, hemicelluloses are also attractive as biopolymers, which can be utilized in their native or modified forms in various areas including food and nonfood applications. Moreover, agricultural straw like wheat straw, rye straw, barley straw, rice straw, oat straw, maize stems, CC, sugarcane bagasse and so on are always available and interesting raw-products. So the purpose of this part is to present an overview about the modification of hemicelluloses from straw and their utilization.

### 4.6.1 The Potential Modification of Hemicelluloses

Hemicelluloses consist of various different sugar units, arranged in different proportions and with different substituents [32, 154, 155]. For example, wheat straw contains various branches such as arabinose, xylose, and uronic acid. The main polysaccharide chain consists of D-xylopyranose units linked by a glycosidic β (1→4) bond. The side chains are formed by L-arabinofuranose units linked by α (1→3) bond. Uronic acid, mainly MeGlcA, is bound to D-xylose



units of the main chain by  $\alpha$  (1 $\rightarrow$ 2) bond [3]. Although hemicelluloses are widely distributed in straw, hemicelluloses utilization is nevertheless limited because of their structural diversity, as depending on their botanical origin and isolation procedure applied mentioned earlier in Sections 4.3 and 4.4. The variability in sugar constituents, glycosidic linkages and structure of glycosyl side chains as well as two reactive hydroxyl groups at the xylose repeating unit of the main chain of hemicelluloses offer various possibilities for regioselective chemical and enzymatic modifications, e.g., partial hydrolysis, graft polymerization, oxidation, reduction, etherification, or esterification of the hydroxyl groups and cross-linking. Functionalization creates novel opportunities to exploit the various valuable properties of hemicelluloses for previously unconceived applications.

For polysaccharides containing exclusively hydroxyl groups, the modification reactions preferably occur at primary OH groups. A pronounced reactivity is observed for the OH groups adjacent to the glycosidic linkage, because of electronic reasons. Consequently, for (1 $\rightarrow$ 4) and (1 $\rightarrow$ 3) linked polysaccharides, e.g., starch and cellulose, the rate of esterification is usually in the order of position  $6 > 2 > 3$  [319]. For polysaccharides with no primary OH groups, esterification or etherification at position 2 is the fastest conversion. Xylans shows a reactivity of the OH moieties in the order  $2 > 3$ . Therefore, modification occurs generally at position 2 and position 3 on xylans. Reaction paths lead to alternative patterns of esterification, etherification, graft polymerization, and other reactions. Modification is useful to add or modify functionality. Recently, cellulose, starch, and their derivatives have received more attention while the hemicelluloses have got, comparatively, little attention. Some current important modifications of hemicelluloses from straw are summarized below.

## 4.6.2 Chemical Modification and its Application

Hemicelluloses, like cellulose, has an abundance of free hydroxyl groups distributed along the backbone and side chains and is, therefore, an ideal candidate for chemical functionalization [155]. The hydroxyl groups of hemicelluloses have been utilized in chemical reactions to produce a variety of new functional groups, which in turn can be utilized in further reactions. A wide variety of possible chemical structures are obtained by chemical modification of hemicelluloses through esterification, etherification, graft polymerization, or oxidation. By forming hemicellulose derivatives through functionalizing available hydroxyl groups, properties such as crystallinity, solubility, hydrophobicity, or hydrophilicity may be modified. The main goal of this article is to review some aspects of the chemistry of straw hemicelluloses. Emphasis is placed on

ethers and esters, which have been a recent focus of interest. The following section mainly discusses the routes of chemical modification and their derivatives' uses, which are used in researches and industries.

### 4.6.2.1 Esterification of Straw Hemicelluloses

The impetus for the increased interest in the synthesis of functionalized natural polymers, in particular esters of hemicelluloses, is their easy biodegradability and conformity to the principles of green chemistry. Recently, in comparison with other chemical modifications of hemicelluloses, more attentions have been paid on the exploitation of hemicelluloses esters because the esterification is a possible path to achieve thermoplastic hemicellulose-based materials. Other reasons for the esterification are to reduce the water absorbency of the hydrophilic hemicelluloses, to increase solubility, and to reduce crystallinity.

The esterification of polysaccharides with carboxylic acids and carboxylic acid derivatives is among the most versatile transformations of these biopolymers. It gives ready synthetic access to a wide range of valuable products [11]. The first report about the hemicelluloses acetates was published as early as 1950s [320]. The author stated that alkaline hemicelluloses, which had high  $M_w$ , were esterified with acetic anhydride in the presence of 0.25% of nitric acid. The acetate was soluble in dioxane, pyridine, or in a chloroform methanol (9:1) mixture. These acetates produced cloudy films, indicating the immiscibility of hemicelluloses acetate with either of the other two acetates. Hemicelluloses acetates with low  $M_w$  from CC were responsible for cloudy films, which meant that these acetates could be cast into good films. In the following years, many hemicellulosic esters have been described [321–323], although the modified hemicelluloses were mostly obtained from wood. Because of shortage of fuel fossil, sustainable resource becomes indispensable for the need of society. Recently, more interests have been focused on the modification of straw hemicelluloses. Straw hemicelluloses acetates prepared under typical conditions used for polysaccharide esterification are completely summarized in Fig. 4.29. The required modification may involve reactions conducted in a homogeneous or a heterogeneous medium. Thus, the following sections are separately discussing the heterogeneous esterification of hemicelluloses and the homogeneous esterification during the past two decades.

Heterogeneous conditions are always unavoidable, whenever surface characteristics of a product are to be modified without affecting its interior. Thickness of the modified layer may be adjusted by controlling the diffusion parameter or by using low-energy radiation which do not penetrate into the bulk of material. The preparation of the acetate, propionate, and butyrate of lima bean pod hemicelluloses and the acetate, propionate, butyrate,



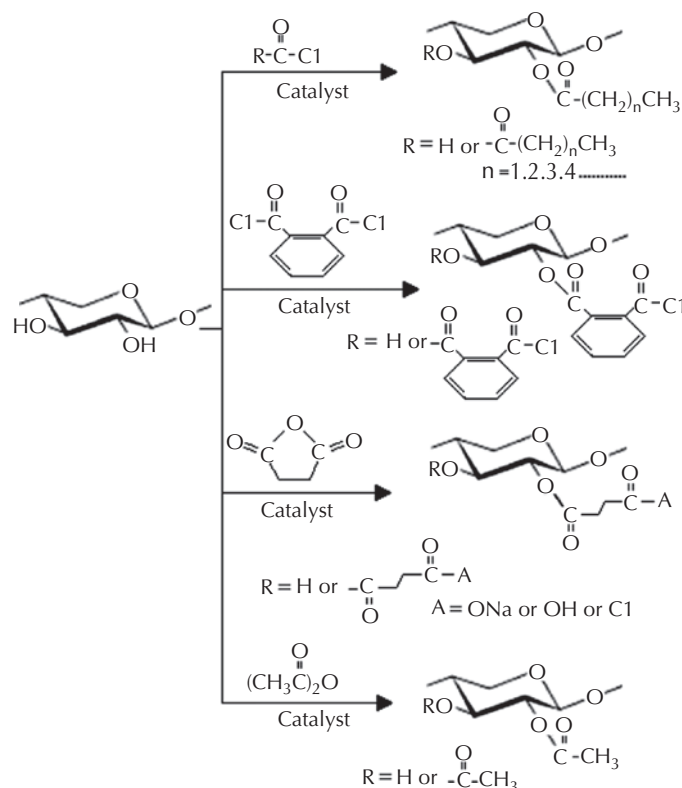


FIGURE 4.29 Introduction of ester groups into xylan rich hemicelluloses.

caprate, laurate, myristate, palmitate, and benzoate of CC hemicelluloses were prepared as early as in 1948 [324]. The esters, except the benzoate, were prepared by reaction with pyridine and acid anhydride or acid chloride under relatively mild conditions. It was found that all of the esters had very low solubility in organic solvents. Heterogeneous esterification of wheat bran hemicelluloses with excess octanoyl chloride (without solvent) was described by Thiebaud and Borredon [325]. The reaction occurred at 25–130 °C for 1–5 h. It was stated that pure hemicelluloses from CC were more reactive and more readily hydrolyzed than oak wood sawdust toward the fatty-acid chloride. The separated liquid fraction contained, as well as lignin, degraded esterified hemicelluloses because of the acid attack and esterification of polysaccharides. The authors also stated that the use of pyridine as solvent in this esterification was found to limit this acid hydrolysis. AX acetate was prepared by Buchanan et al. [10]. Treatment of AX from corn fiber with C<sub>2</sub>–C<sub>4</sub> aliphatic anhydride (butyric anhydride, propionate anhydride, and butyric anhydride) using methanesulfonic acid (MSA) as a catalyst or trifluoroacetic anhydride as a promoter at 35–65 °C for 0.5–3 h resulted in the corresponding biopolymer esters. The AX esters with DS from 1.58 to 2.33 were obtained as high *M<sub>w</sub>*, amorphous solids with glass-transition temperatures ranging from 61 to 138 °C. Additionally, the *T<sub>g</sub>*'s of the AX esters are highly dependent on the DS and

substituent type. The AX esters are thermally stable to near 200 °C but undergo significant and rapid thermal degradation when heated above the onset of thermal degradation. The AX esters are soluble in a range of solvents and in organic solvent/water or MeOH mixtures. Using AX esters and cellulose acetate blends in acetone/2–5 wt% H<sub>2</sub>O could form novel composite.

In the last decade, the chemical modifications of hemicelluloses that carried out in heterogeneous and gel-like phase obtain low DS [323]. To improve the properties of hemicellulosic derivatives, it is necessary to find suitable reaction media that could make derivation reactions to happen in homogeneous phase, and thus, the substitutions along the hemicellulosic backbone can be achieved with satisfactory yields and minor depolymerization of the hemicellulosic chains. Acetylation of the hydroxyl groups of hemicelluloses to increase hydrophobicity is one approach toward increasing the water resistance of hemicelluloses. Derivatization of hemicellulosic hydroxyl groups may also reduce the tendency of hemicelluloses to form strong hydrogen bonded networks and increase film flexibility. Sun et al. [201] reported that hemicellulose acetates were prepared under homogeneous reaction in the system *N,N*-dimethylacetamide/lithium chloride by reacting the native hemicelluloses from wheat straw with acetic anhydride in the presence of 4-dimethylaminopyridine (DMAP) for 72 h at 60–85 °C. At a prolonging period of

72 h, a significant degradation and hydrolysis of the product appeared as showing by the much lower DS (0.84) and  $M_w$  (22, 890 g mol<sup>-1</sup>). Under an optimum reaction condition (85 °C, 60 h), over 80% hydroxyl groups in native hemicelluloses were acetylated. In addition, it was also found that the thermal stability of hemicellulosic acetates were higher than the native hemicelluloses. Moine and coworkers [326] also reported DMA/LiCl as solvent in esterification maize bran heteroxylan. Lauroyl chloride was used to introduce esters on the hydroxyl groups of the xylans by a microwave-activated reaction in DMA/LiCl catalyzed by DMAP. The author stated that 108 and 172% mass ratios were obtained for the dodecyl-grafted xylan and heteroxylan. The degrees of substitution were 1.3 (maximum 2) for xylan and 1.2 (maximum 2.1) for heteroxylan. The resulting products could be used as hydrophobic films.

Strongly polar aprotic solvents such as DMF were found to be able to prevent the aggregation of flexible hemicellulose chains, promoting the interactions between substrate and reagents. Therefore, the DMF/LiCl solvent was used for acetylation of hemicellulose fractions isolated from wheat straw in the presence of DMAP as a catalyst at 60–85 °C for 2–60 h [202]. Hemicellulosic esters with DS from 0.59 to 1.25 were obtained depending on the reaction condition. Under an optimum condition (85 °C, 60 h), approximately 75% of the free hydroxyl groups in hemicelluloses were acetylated. The author also stated acetylated hemicelluloses with low DS are soluble in DMSO and DMA, whereas sample with high DS can be dissolved in hexane or CCl<sub>4</sub> only. It is presumed that there is no solvent for the whole DS range in case of acetylated hemicelluloses. Thus, the soluble hemicelluloses acetates can form film and membranes from the solution by casting and evaporating of the solvent. GPC measurements show that the polymer degradation is low at reaction temperatures of 60–85 °C. Increasing temperatures and prolonged reaction times lead to a significant degradation. Moreover, the acetylated hemicelluloses were found to be more thermally stable than the nonesterified material. Furthermore, under moderate reaction conditions, acylated derivatives with DS ranging from 0.18 to 1.71 were prepared in DMF/LiCl from wheat straw hemicelluloses [203, 327–329], SCB hemicelluloses (DS from 0.04 to 0.89) [330], and rye straw hemicelluloses (DS from 0.37 to 1.65) [57] with stearoyl chloride or other various acyl chlorides. It was found that only a minimal degradation of the macromolecular hemicelluloses occurred during this esterification under moderate condition. The thermal stability of the products decreased slightly upon chemical modification, but no further significant decrease in thermal stability was observed for DS ≥ 0.29 [57]. In addition, the modified hemicelluloses exhibited increased hydrophobic character because of the incorporation of long alkyl groups. However, to give fast, uniform heating and enhancing reaction time, microwave heating, as an alternative to conventional

heating technique, has been proved to be more rapid and efficient [331]. Esterification of rich xylan from wheat straw with acetyl chloride, propionyl chloride, n-octanoyl chloride, lauroyl chloride, palmitoyl chloride, stearoyl chloride, oleoyl chloride, phthaloyl dichloride, and succinyl chloride in DMF/LiCl under microwave irradiation was studied [332–334]. Under an optimum reaction condition (molar ratio of xylose unit in hemicelluloses/lauroyl chloride [1:3], molar ratio of xylose unit in hemicelluloses/triethylamine [1:2], 5% DMAP, 78 °C, 5 min), a DS of 1.63 was obtained quickly and efficiently by microwave [332]. Microwave irradiation was a high efficiency way for the esterification of hemicelluloses, such as lauroylation, acetylation, phthaloylation, and succinoylation [332–334]. It improved the speed of esterification reaction remarkably and made reaction processing smoother. Thus microwave irradiation is advantageous over conventional heating for the hemicellulose esterification reactions. However, microwave irradiation led to a partial degradation of the polymer and, therefore, resulted in a slight decrease in thermal stability of hemicellulosic derivatives in comparison with conventional heating technique [332]. Those prepared hemicelluloses esters, which were biodegradable and thermoplastic, could replace the nonbiodegradable plastic in the plastic industry.

Another method to prepare the acetylated hemicelluloses from SCB in homogeneous system was brought forward by Sun et al. [335]. Hemicelluloses were firstly dissolved in hot water, and then, little amount of DMF was added to form a homogenous system. The water and some amounts of DMF were removed under reduced pressure, and then acetic anhydride and *N*-bromosuccinimide (NBS) were added. The homogeneous reaction mixture was formed and the esterification occurred. Acetylation between DS 0.27 and 1.15 could be prepared by varying the reaction temperature and duration (at 18–80 °C, for 0.5–5.0 h) using 1.0% NBS as a catalyst. The new biopolymer acetates were thermally stable to more than 200 °C but underwent significant and rapid thermal degradation when heated above the onset of thermal degradation.

The succinoylation to introduce carboxylic functionality into polysaccharides is known to increase the hydrophilicity of the material. Succinylation can be used as an effective way to increase the hydrophilicity of hemicelluloses. To obtain hemicellulosic esters containing carboxylic groups, a method of succinoylation has been developed. Sun et al. [333, 336–338] investigated the succinylation of hemicelluloses isolated from wheat straw and SCB reacted with succinic anhydride in DMF/LiCl. It was found that variations in reactant molar ratio from 1:1 to 6:1 and reaction time from 1 to 12 h resulted in an increase in a DS from 0.28 to 1.03 and 0.87 to 1.67, respectively. However, it should be noted that the reaction efficiency decreased as the molar ratio of anhydride to hemicelluloses increased from 6:1 to 8:1. Also, extending the reactions beyond 12 h

at 80 °C led to products of lower substitution [337], as using pyridine as catalyst in DMF/LiCl system. The thermal stability of the products decreased slightly on chemical modification, but a thermal stability of the succinylated hemicelluloses to more than 200 °C is satisfactory. Sun et al. [339] also described the reaction of wheat straw hemicelluloses with succinic anhydride in aqueous alkaline systems. The effects of different reaction times (from 0.5 to 16 h), temperatures (from 25 to 45 °C), and molar ratios of succinic anhydride to anhydroxylose units in wheat straw hemicelluloses (from 1:5 to 1:1) on the DS were studied. The highest DS (0.2) in the pH range 8.5–9.0 was obtained in the temperature range 25–28 °C, at times of 1–2 h, and a 1:1 molar ratio. Despite the low function, the thermal stability of the products was increased, and applications as binders and thickening agents in foods, tablet disintegrants in pharmaceuticals, surface-sizing agents, and coating binds in paper industry are proposed.

Recently, public attentions have been focused on the use of ILs as eco-friendly reaction media because ILs can offer a potentially clean method for carrying out chemical reactions or processes [340, 341]. ILs was firstly reported to be used in the acetylation of wheat straw hemicelluloses by Ren et al. [204]. It was found that hemicelluloses are completely soluble in ILs at 90 °C for 1.5 h up to 2.6% by weight. Acetylated hemicelluloses with DS between 0.49 and 1.53 are accessible to be obtained in a complete homogeneous procedure by varying the condition of reaction. The preferred reaction parameters that resulted in the highest DS of 1.53 were as follows: 20:1 reactant molar ratio; 100 °C; 30 min; and 15% iodine, in which about 83% hydroxyl groups in native hemicelluloses were esterified.

This method has obvious advantages in that the reaction is more rapid and complete, and ionic liquid can be recycled to use. Based on IL's successful application in the acetylation of hemicelluloses, preparation of other hemicellulosic derivatives including hemicellulosic esters with different aliphatic and aromatic acids is expected to be investigated potentially. In summary, Table 4.7 summarized homogeneous esterification of hemicelluloses in different solvents.

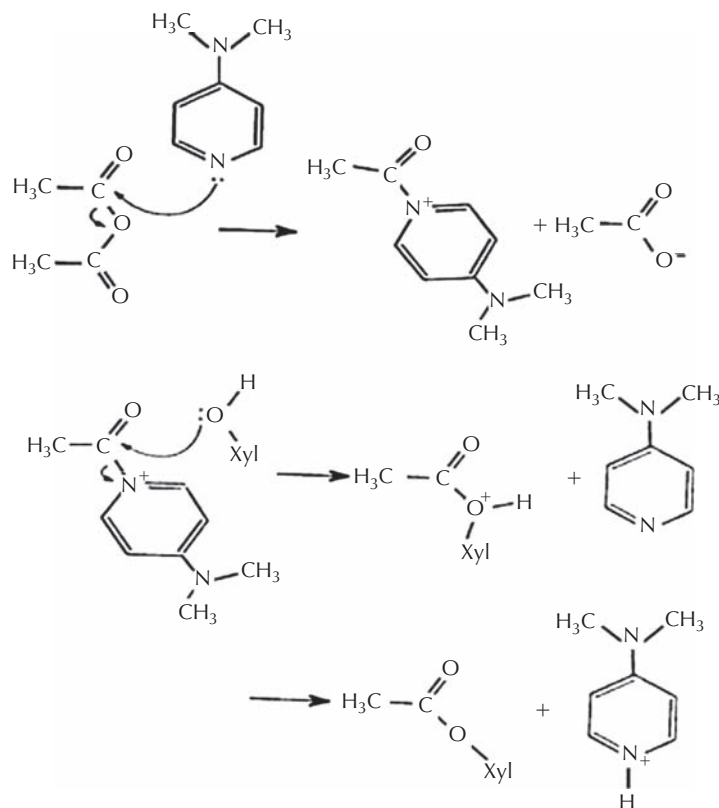
However, the classical method for preparing hemicellulosic esters entails the use of base catalysis and polar solvents such as DMF [324, 202, 342] and DMSO [321, 322]. In this case, the use of a solvent, which reduces the reaction rate because of dilution of modifiers, would require complicated separation procedures to recover the chemicals after the reaction. Organic solvents are often harmful to humans and the environment. Therefore, it is best to eliminate organic solvents in the reaction system [343]. However, an addition of catalysts has been proven to accelerate the reaction rate of acetic anhydride with polysaccharides because the reaction is acid- or base catalyzed [344]. There are various catalysts used in esterification such as H<sub>2</sub>SO<sub>4</sub>, pyridine, NBS, *N*-methylpyrrolidine (MPI), *N*-methylpyrrolidinone (MOP), and DMAP, which have been used to accelerate the esterification such as acetylation [202], succinylation [337, 338], oleoylation [330], and lauroylation [332]. Pyridine-catalyzed acetylation is a standard method for the determination of hydroxyl compounds and other acylable substances. The mechanism involves nucleophilic catalyst with the intermediate formation of the acylpyridium ion [345]. Although pyridine is an effective catalyst in such acylations, it is toxic, has an

**TABLE 4.7** Different Solvent in Homogeneous Esterification of Hemicelluloses Obtained from Different Straws

Solvent	Plant source and reference	Esterification	Agent
DMF/LiCl	Sugarcane bagasse [338]	Succinylation	Succinic anhydride
	Wheat straw [336, 337]		
DMF/LiCl	Wheat straw [327]	Stearoylation	Stearoyl chloride
DMF/LiCl	Wheat straw [332, 329]	Lauroylation	Lauroyl chloride
DMF/LiCl	Wheat straw [202]	Acetylation	Acetic anhydride
DMF/LiCl	Sugarcane bagasse [330]	Oleoylation	Oleoyl chloride
DMF/LiCl	Wheat straw [333, 203]	Acylation	Acylated chloride with different long chain
	Rye straw [57]		
DMA/LiCl	Wheat straw [201]	Acetylation	Acetic anhydride
	Maize bran [326]	Lauroylation	Lauroyl chloride
Ionic liquid	Wheat straw [204]	Acetylation	Acetic anhydride
In aqueous alkaline systems	Wheat straw [339]	Succinylation	Succinic anhydride

unpleasant odor, and is not suitable for use in large-scale reactions [346]. Recently, it has been reported that DMAP is an effective catalyst of analytical acylations by acetic anhydride having a specific catalytic activity about  $10^4$  times greater than that of pyridine [347]. Sun et al. [327, 203] attempted to study the function of DMAP in the esterification of hemicelluloses from wheat straw. In this esterification of xylan-rich hemicelluloses from wheat straw reacting with various acyl chlorides ( $C_3$ – $C_{18}$ ), the author surprisingly found that about 95% hydroxyl groups in hemicelluloses were esterified by the dosage of DMAP of 1.7% (weight based on hemicelluloses) [203]. It was also found that DMAP, a widely used versatile hypernucleophilic acylation catalyst, was  $10^4$  times more active than the pyridine during esterification. And it was also found that triethylamine (TEA) is more effective than pyridine as an acid acceptor to gain high yield and DS. The mechanism of DMAP to celebrate the esterification of hemicelluloses was shown in Figs. 4.30 and 4.31. The catalytic efficiency is probably because of the stabilization of the acyl pyridinium ion, which plays an important role in the catalytic cycle. Steric effects, the donor ability of the amine substituent, and the good nucleophilic properties of DMAP additionally affect the reactivity [348–350]. Although it is so effective to accelerate the reaction rate, DMAP is too expensive to be commercially applied. An interesting new catalyst usable for hemicelluloses esterification with acetic

anhydrides is NBS. The inexpensive and commercially available reagent shows high efficiency for the catalysis of the acetylation of hemicelluloses with the corresponding anhydride [335]. A DS of 1.15 is obtained after 2 h at  $80^\circ C$ , with 1% NBS as catalyst. The actual role of NBS is not clear, but there is a plausible explanation that NBS might act as a source for  $Br^+$ , which in turn activates the carbonyl groups of acetic anhydride to produce the highly reactive acylating agent  $[CH_3-CO-N-(OCCH_2CH_2CO-)]$ . This acetylating agent reacts with hydroxyl groups of hemicelluloses, which upon elimination of NBS produces acetylated hemicelluloses ( $Xyl-O-CO-CH_3$ ) (Fig. 4.32). Aside from DMAP and NBS as high efficient catalyst in the esterification of hemicelluloses,  $I_2$  was found to be used as a novel catalyst in the acetylation of wheat straw hemicelluloses with acetic anhydride in ILs [204]. Comparing with hemicelluloses esters without addition of catalyst (DS, 1.32), higher DS up to 1.53 with addition of catalyst (15%, weight based on hemicelluloses) could be obtained under the same condition. The actual role of iodine in this acetylation is not clear, but there is a plausible explanation that iodine might be ionized into  $I^+$  and  $I^-$  in IL, and  $I^+$  in turn activates the carbonyl groups of acetic anhydride for further reaction. The roles of iodine in IL maybe just like NBS as catalyst in the esterification of hemicelluloses mentioned in previous articles [338, 351]. In addition, MSA also was used as a catalyst in the



**FIGURE 4.30** The mechanism of dimethylaminopyridine (DMAP) to celebrate the esterification of wheat straw hemicelluloses.

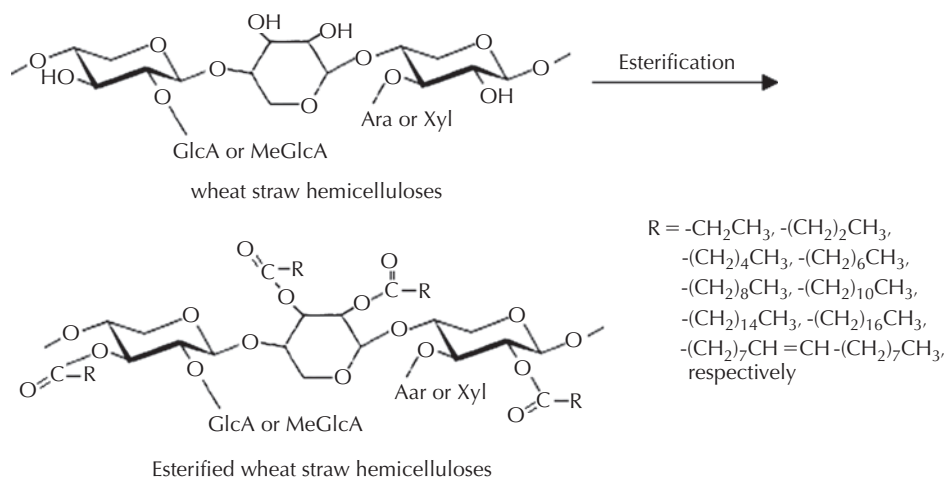


FIGURE 4.31 Esterified wheat straw hemicelluloses [203].

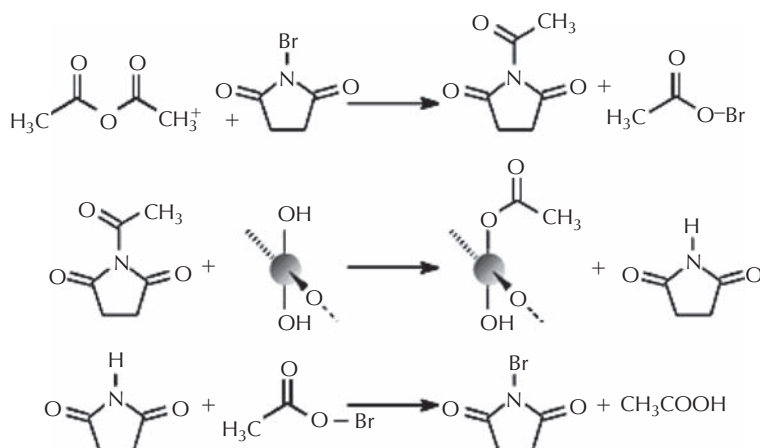


FIGURE 4.32 Proposed mechanism for the acetylation of hemicelluloses with *N* bromosuccinimide (NBS) as catalyst [335].

esterification of corn fiber AX reacted with  $C_2$ – $C_4$  aliphatic anhydrides [10].

Besides the esterification reaction mentioned earlier, the sulfation, tosylation, nitration, and xanthation belong to the esterification. The sulfation [352, 353], tosylation [354], nitration [355], and xanthation [356] of hemicelluloses from wood have been investigated widely, whereas they have not been used in the chemical reaction of the straw hemicelluloses yet. Confidently, more researches will be focused on those reactions of hemicelluloses from straw in future.

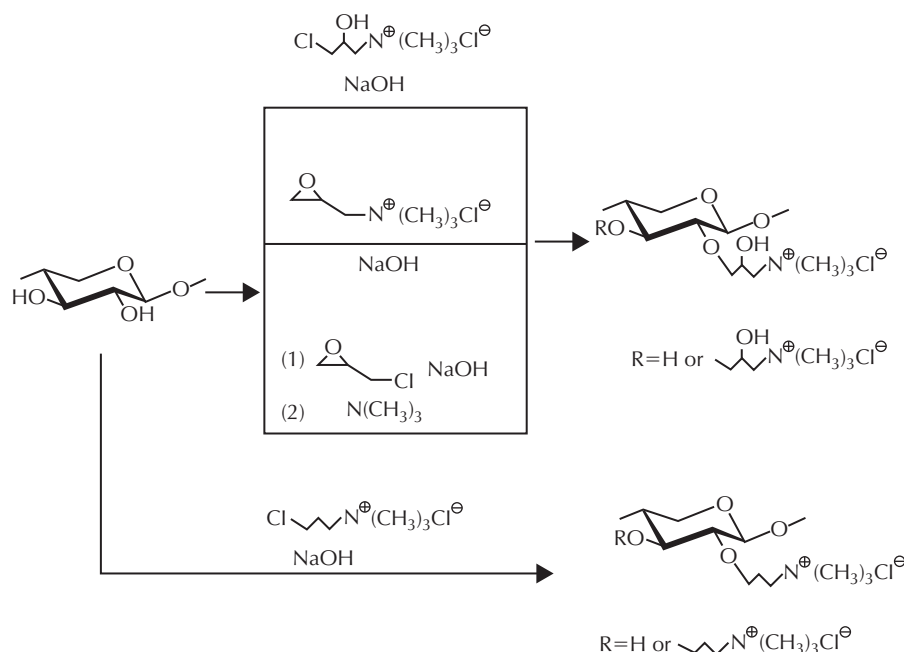
#### 4.6.2.2 Etherification of Hemicelluloses

Etherification of hemicelluloses is important for increasing solubility, stability against microorganisms to prevent fermentation on storing, film forming ability, and increasing viscosity. Many different kinds of reactions, such as carboxymethylation, methylation, quaternization, benzylolation, and sulfoalkylation and so on, belong to etherification.

An overview of reported hemicellulosic ether forms is given as below.

Similar to cellulose or starch, cationic groups are introduced into the chain of hemicelluloses from straw by the formation of ether bonds in various reagents (Fig. 4.33). Mostly, the polymer is reacted with prebuilt cationic reactants bearing halogens or epoxide rings as reactive moieties. Quaternization of hemicelluloses enhances their solubility and yields cationic or ampholytic polymers which have similar chemical properties to quaternized derivatives obtained from starch, cellulose, and chitosan [357–359]. Recently, more and more attention and interests have been paid to the researches in this field, especially, the quaternization of hemicelluloses [360]. Investigations were focused on xylan-rich waste materials such as SCB [361], CC [362], and beech sawdust [363], and the quaternized derivatives were prepared by reacting with 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHMAC) or 2,3-epoxypropyltrimethylammonium chloride





**FIGURE 4.33** Reaction pathways for the introduction of cationic moieties into xylan.

(ETA). Subsequent extraction steps with water and dilute alkali resulted in fractionation into trimethylammonium-2-hydroxypropyl (TMAHP) cellulose, TMAHP-hemicelluloses, and lignin. TMAHP-hemicelluloses are useful as beater additives [364]. Recently, the quaternization of xylans, isolated from CC, rye bran, and beech wood was investigated [199]. The reaction was carried out with a NaOH-activated xylan and CHMAC as alkylating reagent. The results clearly showed that the DS depended on the molar ratios of CHMAC/xylan and NaOH/CHMAC, as well as the xylan type used. In addition, it was also found that hot water pretreatment of the xylans in the alkaline activation step significantly enhances their reactivity. The author also investigated the applicability of quaternized xylans as a retention aid in TMP. The results indicated the potential of quaternized xylans as cationic polymer additives. The cationized xylans with DS 0.25–0.98 were characterized by  $^{13}\text{C}$ -NMR spectroscopy after the hydrolytic chain degradation [365]. Xylans were converted into TMAHP-hemicelluloses by treating the NaOH-activated polymer with CHMAC. It was found that monosubstitution of the xylose units was found mainly to occur in the studied DS range. And at lower DS, substitution prefers to happen at OH-2 groups, whereas at higher DS there is no regioselectivity observed. The TMAHP derivatives prepared from isolated xylans improved the strength properties of bleached hardwood kraft pulp and unbleached thermomechanical spruce pulp, and they can increase the retention of fines on fibre surface in paper sheets [365]. Additionally, the cationic xylan derivatives exhibit antimicrobial activity against some Gram-negative and Gram-positive bacteria, depending on the DS and xylan type [366]. The authors

stated the macromolecular backbone of the derivatives played an important role in the expression of their antimicrobial activity. Also the neutral and/or acidic glycosyl side chains of the quaternized xylan polymer may be involved in the mechanism, contributing to the intermolecular interactions. These derivatives may be useful in medical application. The introduction of TMAHP groups also affected the rheological properties of the xylan chains [367]. At high DS (>0.5), the former shear-thinning xylans became dilatant, probably as a result of strong inter and intramolecular interactions. Ren et al. [368–372] attempted to investigate quaternization of the xylan-rich hemicelluloses isolated from SCB by reacting with ETA or CHMAC in different media. Comparing the different media such as aqueous alkaline solution, ethanol/water, DMSO, it was found that the highest DS value of 0.55 of cationic hemicelluloses was synthesized in aqueous NaOH solution. However, the  $M_w$  of products prepared in aqueous alkaline solution was much lower than that of the products prepared in DMSO and ethanol/water. This implied that hemicelluloses polymers were substantially degraded in aqueous NaOH. Interestingly, hemicelluloses were only slightly degraded in DMSO compared with other derivatization procedures. Those polymers derived from SCB hemicelluloses were used as dry strength agent in papermaking and could obviously improve the physical properties of sheets from BKP and OCC pulp [289–291].

Carboxymethylation is one of the most versatile functionalized procedures, and it is an effective way to prepare biological materials because it endows polymer materials many valuable properties, such as inspissations, filming, emulsification, suspension, water maintaining and binding,

etc. [373]. Thus carboxymethylated polymers have been used for many applications, such as printing and dyeing, medicine, food, textures, toilet, oil drilling, electrical elements, papermaking, etc. Many polysaccharide derivatives have been prepared by carboxymethylation reactions by using substances such as cellulose [374], starch [375], and cashew tree gum [376]. Carboxymethyl hemicelluloses (CMH), the derivatives of hemicelluloses, have better solubility in water than the natural hemicelluloses and have hydroxyl and carboxyl functional groups as well. It is derived as sodium salts by means of interaction of alkaline hemicelluloses with monochloroacetic acid or its sodium salt (Fig. 4.34). Simultaneously, the reaction with sodium monochloroacetate hydrolysis leads to the formation of sodium salts of glycolic acid [377]. In comparison with carboxymethyl cellulose and starch, the carboxymethylation of xylan is investigated insufficiently up to now. In the past, only a few articles described CMH isolated from oats and rice with a DS lower than one [378, 379]. Recently, more attentions have been focused on the carboxymethylation of wood hemicelluloses [380, 381]. Only a few articles reported the CMH from cereal straws. Ebringerova and Hromadkova [85] attempted to investigate the biological activity of hemicelluloses through carboxymethyl modification of CC AGX. The carboxymethylation of CC AGX was performed with monochloroacetic acid in a NaOH/isopropylalcohol medium at 60 °C. A study of the influence of the slurry medium and activation procedures on the carboxymethylation of xylan from oat husk, rye bran, corncob, seed husk (ispaghula), and birch was investigated by Heinze by  $^1\text{H}$  NMR and HPLC [380–382]. It was found that the DS value of carboxymethyl arabinoxylan (CMAX) as high as 1.81 was achieved. CMAX is water soluble starting at DS of 0.33 [382].  $^1\text{H}$  NMR spectroscopy after total hydrolysis revealed carboxymethylation prefers to happen at position 2 compared with position 3. This was because of the well-known higher reactivity of *O*-2. Moreover, position 3 was more occupied by arabinofuranosyl branches and hence less available for carboxymethylation. CMAX showed biological activity and may be used as thickening agent. Xylan-rich hemicelluloses from SCB were converted into CMH by

carboxymethylation using sodium monochloroacetate and NaOH in ethanol/water medium [377]. The product with a maximum DS of 0.56 was obtained at 65 °C for 70 min under two-step reaction of alkaline activation. It was found that a significant degradation of the polymers occurred during carboxymethylation, and the thermal stability of CMH was higher than that of the native hemicelluloses. The optimum conditions for the preparation of CMH from SCB could be exploited to prepare industrial products. The authors also found that the derivatives could be used as wet end additives in papermaking [291], and their function to fibers in pulp is similar to the function of carboxymethyl starch and carboxymethyl celluloses with fibers in pulp.

For any research program aiming at developing novel functional materials, recently Ren et al. [383–385] have firstly investigated hemicellulosic derivatives with bio-functional groups. Those hemicellulosic derivatives containing carbamoylethyl groups and carboxyl groups show new properties, such as hydrophilic character and ampholytes properties. Acrylamide served as the novel etherification reagent, to form the carbamoylethyl ethers by reacting with the hemicelluloses under alkaline conditions on the basis of the Michael addition (a in Fig. 4.35). However, the carbamoylethyl groups could also be easily saponified to carboxylethyl groups with a more heavily alkaline aqueous medium (b in Fig. 4.35). Thus, the derivatives containing bifunctional groups of carbamoylethyl and carboxylethyl are most likely prepared by varying the reaction of conditions, which was confirmed by  $^{13}\text{C}$  NMR and  $^1\text{H}$  NMR. Hemicellulosic derivatives with total DS values of 0.58 (the ratio of carbamoylethyl groups to carboxylethyl groups, 2.5) were obtained by varying the reaction parameters. It was also found that the thermal stability of the resulting products decreased after chemical modification. The products could be used as wet end additives and flocculants in papermaking and sewage-treatment plants. Of course, much more work is required to investigate those hemicellulosic derivatives such as the substituent distribution on the backbone of xylose unit in hemicelluloses, and the solubility in water, zeta potential in distilled water, and

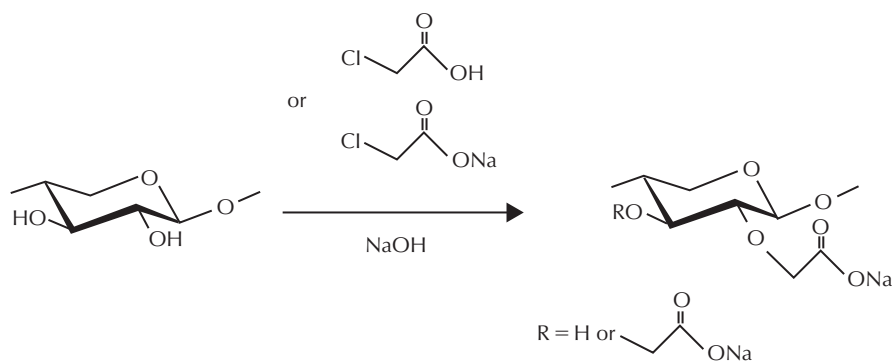


FIGURE 4.34 Reaction scheme of carboxymethylation of xylan rich hemicelluloses.

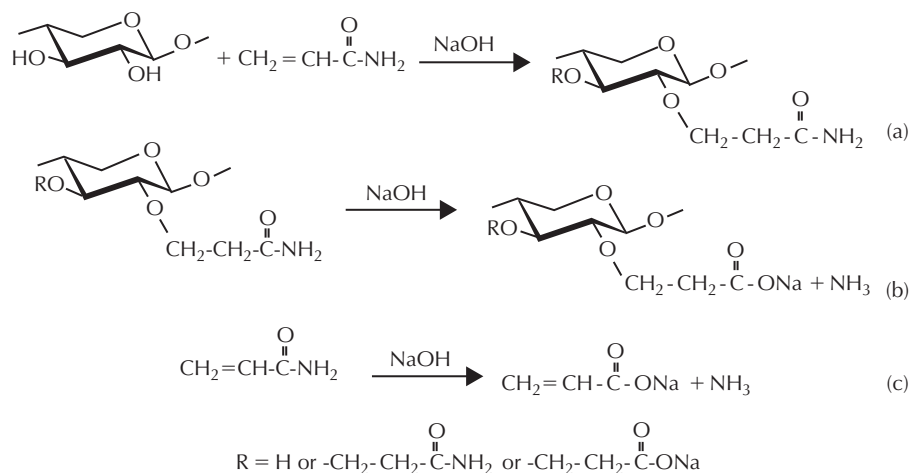


FIGURE 4.35 The etherification of hemicelluloses with acrylamide in alkaline condition [383].

viscosity behavior and so on to further exploit their unknown application to a greater extent.

Methylation of hemicelluloses had already been used in the 1960s as a means of making hemicellulose solvent-soluble for the determination of nonreducing end groups to learn the branching of hemicelluloses [386]. Recently, the methylation of wheat straw hemicelluloses reacted with methyl iodide using sodium hydride as a catalyst reacted in DMSO has been reported [387]. The DS values of 1.7 could be obtained, which meant more than 90% of the free hydroxyl groups in the native hemicelluloses were methylated under the reaction condition given. But the DS were lower than the theoretical maximum of DS of 2. The incomplete reaction is presumed to be because of the following two reasons: first, some of hemicelluloses are degraded in the reaction; second, the hemicelluloses in DMSO may not be fully swollen. And it was found that the thermal stability of modified hemicelluloses significantly increase because large amounts of methyl groups substituents are grafted on the backbone of hemicelluloses by methylation. The methylated hemicelluloses could be used as plastic materials for industry. Furthermore, the development of the methylation reaction makes feasible the use of methyl iodide as the alkylating reagent. The present method has obvious advantages in that the reaction is more rapid and complete when catalyzed by the carbanion and can be controlled by the amount of the reagent added and can be carried out at room temperature in one continuous process without the use of complicated apparatus. The overall chemical reaction was divided into two steps, as shown below:

1. Hemicellulose-OH + CH<sub>3</sub>-SO-CH<sub>2</sub><sup>-</sup>Na<sup>+</sup> → Hemicellulose-O-Na<sup>+</sup> + CH<sub>3</sub>-SO-CH<sub>3</sub>;
2. Hemicellulose-O-Na<sup>+</sup> + CH<sub>3</sub>I → Hemicellulose-O-CH<sub>3</sub> + NaI. The typical reaction mechanism was shown clearly in Fig. 4.36.

Hydroxypropylated polymer is used widely as thermoplastic materials. Jain et al. [388, 389] functionalized xylan from barley husks with propylene oxide in aqueous alkali solution for 24 h at room temperature to yield hydroxypropylated xylan (HPX), which in turn was acetylated with acetic anhydride to produce acetoxypopyl xylan (APX) (Fig. 4.37). Derivatives with different degrees of substitution from 0.2 to 1.9 were prepared by varying the pH of the reaction mixture from 10.5 to 13.0. HPX formed tough transparent films, which were water soluble at low DS (0.2–0.5) and exhibited tensile strengths of 35–45 MPa. In contrast, films cast from APX were soluble in a number of organic solvents. No T<sub>g</sub> was observed for the unsubstituted xylan, whereas an increase in DS of both HPX and APX clearly had a diminishing effect on T<sub>g</sub> because of internal plasticization. Both types of substituted xyans were used as thermoplastic additives in polystyrene (0–20%) revealing a decline in dynamic shear viscosity with increasing xylan content. APX was furthermore injection molded with polystyrene and studied by dynamic thermomechanical analysis (DTMA), whereby the plasticizing properties of the additive were demonstrated. The properties of HPX and APX derivatives qualify this material as a potential biodegradable and thermoplastic additive to melt-processed plastics. Blend characteristics with polystyrene reveal a shear-thinning effect in melt and a plasticization effect in solid state.

Benzylation was developed with benzylbromide derivatives as benzylating agent. This benzylation of xylan from wood has been investigated by Ebringerova et al. [390–392]. The derivatives exhibited remarkable emulsifying and protein foam-stabilizing activities. However, this reaction has not yet been used in the straw hemicelluloses. But, more attentions will be focused on this reaction because of the potential application of benzylated hemicelluloses in many industries.

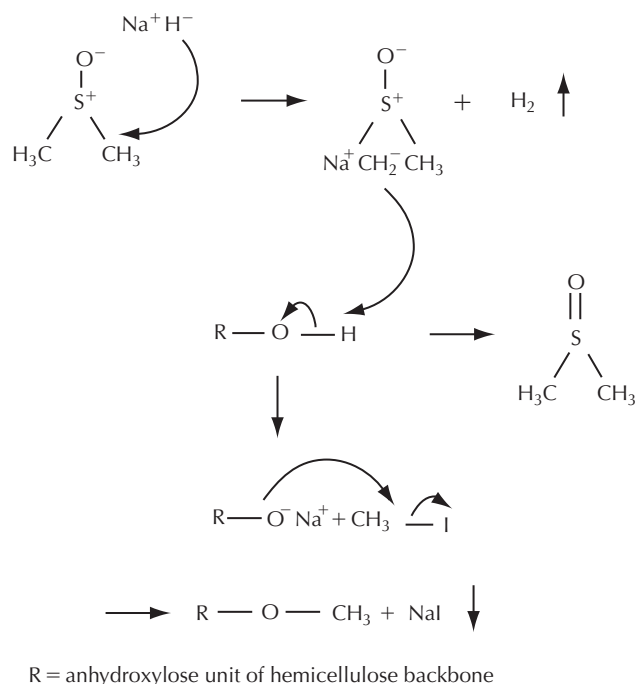


FIGURE 4.36 Scheme of mechanism of catalyst in methylation [387].

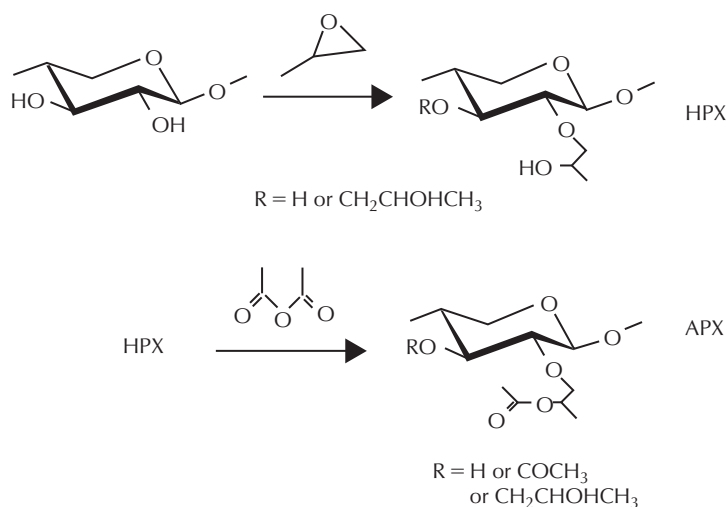


FIGURE 4.37 Conversion of xylan to hydroxypropyl xylan (HPX) and further to acetoxypropyl xylan (APX) [388].

#### 4.6.2.3 Graft Polymerization of Hemicelluloses

Grafting is another technique that has received considerable attention lately for modifying polysaccharides [393]. It is a useful method, not only to improve some of the original properties of polysaccharides but also to introduce new functionality into copolymers products. The free hydroxyl groups in hemicelluloses could be used as initiator sites to perform polymerizations of a number of monomers to form grafts [394]. However, the graft copolymerization with hemicelluloses substrate has received comparatively little

attention, compared with the graft copolymerization with celluloses [395, 396]. An overview of substrates, monomers, and initiators is given, in Table 4.8.

Fanta et al. [397] and El-Shinnawy and El-Kalyoubi [398] independently investigated the graft copolymerization of acrylonitrile onto hemicelluloses from wheat straw and bagasse using ceric ammonium nitrate as an initiator and stated that the graft yield depends on the monomer and initiator concentration as well as reaction time and temperature. The resulting hemicellulose-g-polyacrylonitrile (PAN) copolymers were fractionated by extraction at

**TABLE 4.8** Overview of Substrate, Monomers, and Initiators

Plant source	Grafted monomer	Initiator	Ref.
Wheat straw	Acrylonitrile and methylacrylate	Ceric ammonium nitrate/nitric acid and ferrous sulfate/H <sub>2</sub> O <sub>2</sub>	397
Bagasse	Acrylonitrile	Ceric ammonium nitrate/nitric acid	398
Corn cobs	Acrylonitrile	Potassium permanganate/sulfuric acid	399
Maize bran	Stearyl acrylate and stearyl methacrylate	Oxygen plasma/electron beam irradiation	400

room temperature with dimethylformamide and DMSO. Saponification of the PAN component of hemicellulose-g-PAN gave a water-dispersible graft copolymer with good thickening properties to water systems. An absorbent polymer, similar to the starch-based absorbents (Super Slurpers), was produced when saponified hemicellulose-g-PAN was isolated by methanol precipitation and then dried. Although, ceric ammonium nitrate could be used as an initiator for graft polymerizations onto low-lignin hemicelluloses, it is not easy to work with wheat straw hemicellulose containing 11% lignin. Thus, ferrous sulfate H<sub>2</sub>O<sub>2</sub> redox system was used to initiate graft polymerizations onto this high-lignin material, and the properties of the resulting hemicellulose-g-poly(methyl acrylate) and saponified hemicellulose-g-PAN graft copolymers were evaluated [397]. Soliman [399] described the ability of potassium permanganate to induce graft copolymerization of acrylonitrile onto hemicellulose obtained from alkali extracted CCs holocellulose. Chemical analysis of the reaction product of hemicelluloses and acrylonitrile in the presence of potassium permanganate revealed that the potassium permanganate acted as an initiator for polymerization of acrylonitrile and as an oxidizing agent for hemicelluloses. The grafted samples were characterized by thermogravimetric analysis. The data obtained showed that the thermal degradation of the samples obeys a first-order reaction. The results, furthermore, indicated that the activation energy increases with grafting. The kinetics parameters indicated that grafting increases the thermal stability of hemicelluloses. Moreover, the grafted CCs hemicelluloses could be used as additives for improving strength properties and water retention values of the paper sheets prepared from wood and bagasse pulp. To obtain high-value products, Peroval et al. [400] attempted a different approach to prepare the arabinoxylan obtained from maize bran films by grafting the monomers stearyl acrylate (SA) and stearyl methacrylate (SM) by electron beam treatment. Three distinct processes were carried out: (1) cold plasma treatment of glycerol-plasticized AX films followed by impregnation with monomer and grafting by electron beam, (2) emulsified films (i.e., films from blends of monomer, glycerol, glycerol monostearate, and AX) were electron-beam treated, and (3) pretreatment of AX

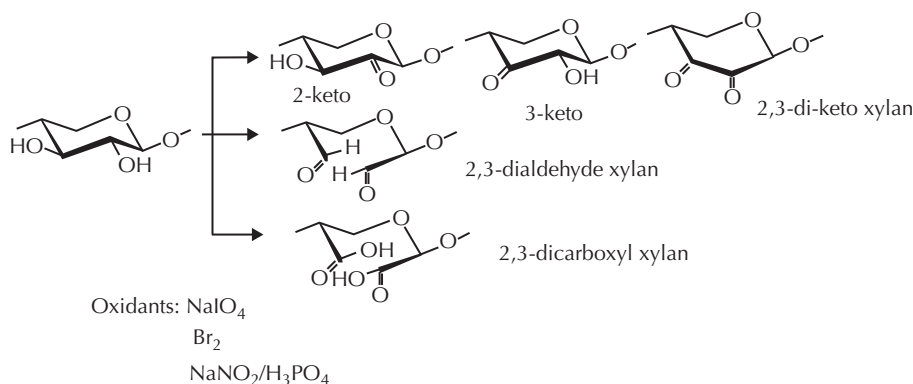
films with electron beam followed by impregnation with monomer and grafting by electron beam. Treatment A was also performed with hydrogenated palm kernel oil (OK35) as monomer, resulting in potentially edible films. Comparison of the films showed that the monomer in all cases induced increased hydrophobicity with static water contact angles increasing from 71° up to a maximum of 123° in the case of an SM-grafted film. Water permeability was decreased with the introduction of the monomers and was, furthermore, dependent on the grafting process. For SA and SM, treatment A was the least effective in reducing permeability. When the preactivation by oxygen plasma is replaced by an electron beam exposure (C treatment), the SA-emulsified films are the most efficient barriers against water vapor developed in this study. To develop biodegradable or edible packaging materials with these grafting procedures, food grade monomers such as polyunsaturated fatty acids should be chosen for further researches.

#### 4.6.2.4 Oxidation of Hemicelluloses

Oxidation is an important tool for the introduction of carbonyl and carboxyl functions into biopolymers. The tendency of the oxidation of polysaccharides depends substantially on the nature of the oxidations and the conditions. Most of the oxidations known from the low-molecular organic chemistry produce both carbonyl and carboxyl functions to a varying extent depending on the experimental conditions. Moreover, even the so-called selective oxidation reactions will result in more or less depolymerization of the macromolecules. In principle, xylan as polyhydroxy compounds bearing secondary hydroxyl groups can be oxidized to 2-keto, 3-keto-, and 2,3-diketo xylan neglecting the transformation of the end groups [360]. Moreover, 2,3-dialdehyde xylan may be obtained in the well-known glycol cleavage oxidation of vicinal diol units with sodium periodates, which may be further oxidized to give 2,3-dicarboxyl xylan. Figure 4.38 summarizes the structures of the possible oxidized repeating units.

Up to now, oxidization has not been applied in the modification of straw hemicelluloses. However, it has been widely used in these raw agriculture residues such as maize bran, jute sticks, OSs, and grass. Ishak and Painter [401]





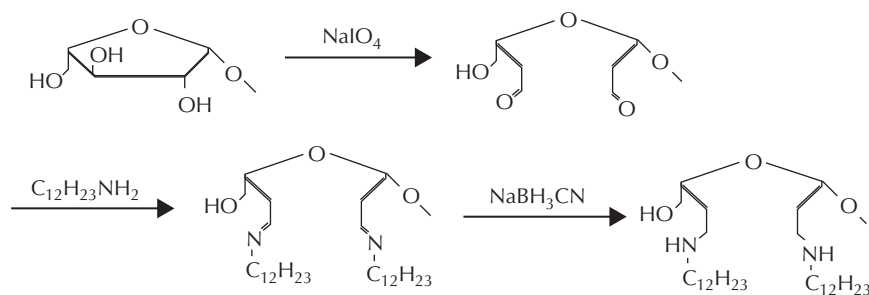
**FIGURE 4.38** Typical repeating units of oxidized xylan.

studied the oxidation of the xylan of *Rhodymenia palmate* with aqueous sodium periodate. They found that the oxidation proceeded in two stages: an initial, rapid stage in which the aldehyde groups form hemiacetal rings with vicinal hydroxyl groups; and a slow, terminal stage, in which the remaining unoxidized units are oxidized. Reductions of the hemiacetals with sodium borohydride allowed the rapid consumption of further periodate to give the oxidation limit. Augustinsson and coworkers [402] reported the oxidation of xylan obtained from retted jute sticks with bromine in the presence of sodium metaborate. It was found that oxidation had occurred at C-2 or C-3. No oxidation of the MeGlcA residues was detected because 4-*O*-methylglucitol was the only hexitol found in the sugar analyses of the carboxyl-reduced oxidized xylans [403]. Xylan from oat apels was oxidized to 2,3-dicarboxylic xylan in a two-step procedure by using  $\text{HIO}_4/\text{NaClO}_2$  as oxidants [404, 405]. Xylan was treated with periodic acid for 6 h at 4 °C to obtain 2,3-dialdehyde xylan with 39 mol % dialdehyde groups. Subsequent treatment with sodium chloride for 24 h at 20 °C and 1 h at 50 °C leads to sodium 2,3-dicarboxylic xylan with 39 mol% dicarboxylate moieties. A set of oxidized samples consisting of dicarboxylate functional groups in the range from 29 to 53 mol% was synthesized by varying the reaction time. These products can be used as biologically degradable detergents. Matsumura et al. also showed the comparison of the biodegradability of oxidized xylan with oxidized amylase by xylanases and  $\alpha$ -amylases on the degree of oxidation [406]. These results indicated that enzymatic degradation occurs if the content of oxidized repeating units was in a range in which the polymers still contained blocks of nonoxidized sugar residues. It was found that the biodegradability of xylan derivatives was higher than that of oxidized amylase but amylase may have higher carboxyl content than xylan to gain enzymatic degradation. The neutral xylan fraction from *Palmaria decipiens* was treated with aqueous bromine solution [407]. The appearance of carbonyl groups was revealed by means of FTIR ( $\nu_{\text{C=O}}$  at  $1741\text{ cm}^{-1}$ ) and NMR spectroscopy. The presence of  $\text{C=O}$

was also revealed chemically by reactions with *p*-chloroaniline or BSA followed by reduction to the corresponding amine with sodium cyanoborane. The oxidation occurs at C-2 only as determined by means of GLC after reductive cleavage, reduction, and peracetylation. Moreover, 2,3-dialdehyde xylan from *Palmaria decipiens* was obtained by the sodium periodate oxidation [408]. The dialdehyde moieties can be further converted into a Schiff base-type compound by the reaction with *p*-chloroaniline. Maize bran heteroxylan was used to produce hydrophobic films by Fredon et al. [409]. Modification entailed oxidation with sodium periodate followed by reductive amination with dodecylamine and sodium cyanoborohydride, which resulted in ring opening and incorporation of dodecylamine on the remaining hemicellulose backbone (Fig. 4.39). The DS of the dodecylamine-grafted xylan films ranged between 0.5 and 1.1, depending on the reaction conditions. The maximal DS was reached under the following experimental conditions:  $\text{NaIO}_4$  2 eq per sugar unit, laurylamine 1.7 eq,  $\text{NaBH}_3\text{CN}$  5.8 eq per aldehyde. The plastic behavior at ambient temperature was correlated with the glass-transition temperature which was around  $-30\text{ °C}$ . The product indicates potential application as a bioplastic [409].

### 4.6.3 Straw Hemicelluloses and Their Application

The hemicelluloses are potentially very useful. As a result of the modification of the various type hemicelluloses from straw summarized earlier, the potential application of these straw polysaccharides is immense and widespread over various technical and non technical fields. Hemicelluloses and their derivatives from straw are applicable as gels, films, coatings, adhesives, and gelling, stabilizing and viscosity-enhancing additives in food and pharmacy as well as in other industrial branches [11]. Studies on utilization of hemicelluloses from cereal straws have demonstrated to be a potential fermentation feedstock in production of ethanol,



**FIGURE 4.39** Oxidation followed by reductive amination of vicinal hydroxyl groups of arabinofuranose to yield dodecylamine functionalized heteroxylan.

acetone, butanol, and xylitol. The current uses of xylan on an industrial scale involve their conversion to xylose, xylitol, and furfural. Xylitol has received much attention. Xylitol is a natural polyol with particular physicochemical properties, which make them useful both in foods like chewing gum, bakery, and chocolate as a sweetener with anticariogenic property, and in medicines as a sugar substitute for the treatment of diabetes and erythrocytic glucose-6-phosphate dehydrogenase deficiency [410]. Xylitol is produced by hydrolysis of xylan, crystallization of xylose, and hydrogenation. Many studies have been conducted utilizing the hemicellulose portion of agricultural residues like eucalyptus [411], rice straw [412], CC [413, 414], brewer's spent grain [415], SCB [416], and corn stover [417] for xylitol production. Moreover, it's valuable to exploit the potential applications of wheat straw hemicelluloses as adhesives, thickeners, stabilizers, and film formers and emulsifiers [69]. Based on an extensive study of hemicelluloses from wheat straw, Sun et al. [418] also found that wheat straw hemicelluloses latexes showed great potential for making decorative paints, which indicated a possibility of using straw hemicelluloses for real commercial decorative paint industry. Evidently, hemicellulosic biopolymers have been widely applied in food and nonfood area. CC xylan, referred as corn fiber gum, is quite a sticky polymer. Therefore, it could serve as adhesive, thickener [69], additive to plastics (due to increasing their stretch, breaking resistance, and makes them more susceptible to biodegradation) [419], food additive (because of its emulsifying activity and ability to stabilize protein foam during heating) [70]. In addition, CC xylan can be used as biopolymer to interact with clay (montmorillonite type clay, NaMt) to form biocomposites [420]. The biocomposites, which showed better thermal and rheologic behaviors with respect to the starting materials, can find application especially in cosmetics formulations, as both thickener and cleaner agent. Sugar (81.16% xylose and 15.27% glucose) from hemicelluloses hydrolysis by the pretreatment of corn stover with dilute sulfuric acid was used to cultivate genetic recombinant *Escherichia coli* BL21 with human-like collagen expression enhanced by 50.00% and 63.71% xylose consumption [307]. In particular, some hemicelluloses from higher plants and herbs represent a

potential source of pharmacologically active polysaccharides. Glucuronic acid-containing (acidic) xylans isolated from annual plant residues such as bamboo leaves, corn stalks, and wheat straw [245] have been reported to inhibit markedly the growth of sarcom-180 and other tumors, probably because of the indirect stimulation of the non-specific immunological host defense [421, 422]. Arabino-(glucurono) xylans isolated from *Echinacea purpurea*, *Eupatorium perfoliatum* have been reported to have immunostimulating effects [423, 424]. Similarly, 4-*O*-methylglucuronoxylan from *Chamomilla recutita* [425] and the acetic, highly branched heteroxylan from *Plantago* species [426, 427] have been found to have antiinflammatory activity.

Because of the shortage of energy resource, more attention has been paid to the utilization of hemicelluloses from straw and other agricultural residents, recently. The potential use of hemicellulose-based or derived products in an industrial and biomedical context is beyond dispute and will stimulate further activities in basic and applied research.

## 4.7 SUMMARY

In summary, functional groups have been successfully introduced to the hydroxyl groups of various hemicelluloses by esterification, etherification, oxidation, graft polymerization, and other reaction. The resulting products have altered properties compared with the parent compounds in terms of hydrophilicity, polyelectrolyte ability, strength, elasticity, hydrophobicity, and thermoplastic properties. Those properties make hemicellulosic derivatives be able to be used in different industries, such as films, coating, pharmaceutical, food, papermaking, and other industries. However, there are still many potential pathways that have not been investigated, and therefore, it can be argued that the application of hemicelluloses in fields such as packaging and biomedicine still hold considerable promise for the future.

By now, the researches of hemicelluloses derivatives are mainly focused on developing advanced materials. It should be pointed out that these researches comply with the

principle of green chemistry because their target is to develop biodegradable products by employing renewable resources as starting materials. Hemicelluloses together with other polysaccharides would become outstanding starting materials of “green” advanced materials if the challenges discussed below are resolved. For example, the solubility of hemicelluloses in simple, nontoxic media; the activation of a modifying reaction by methods that meet greenchemistry principle; the reactions happening during the chemical conversions; and the interaction of polymer backbone with modifying groups (esterification, etherification, oxidation, graft polymerization) have not been completely understood. And the modification of hemicelluloses with natural molecules has not been investigated, although it’s meaningful for preparing pure bio-based materials. In brief, hemicelluloses chemistry will be more and more important both in basic scientific research and applying research area.

## REFERENCES

- [1] A.M. Stephen, Other plant polysaccharides, in: G.O. Aspinall (Ed.), *The Polysaccharides*, Vol. 2, Academic, New York, 1983, pp. 97–193.
- [2] T.M. Schindler, The new view of the primary cell wall, *Zeitschrift für Pflanzenernährung und Bodenkunde* 161 (1998) 499–508.
- [3] R.C. Sun, J.M. Lawther, W.B. Banks, Fractional and structural characterization of wheat straw hemicelluloses, *Carbohydr. Polym.* 29 (1996) 325–331.
- [4] A. Ebringerova, T. Heinze, Xylan and xylan derivatives biopolymers with valuable properties. 1. Naturally occurring xylans structures, isolation procedures and properties, *Macromol. Rapid Commun.* 21 (2000) 542–556.
- [5] J. Puls, B. Saake, Industrially isolated hemicelluloses, in: P. Gatenholm, M. Tenkanen (Eds.), *Hemicelluloses: Science and Technology*, ACS Symposium Series 864, American Chemical Society, Washington, DC, 2004, pp. 24–37.
- [6] K.W. Waldron, M.L. Parker, A.C. Smith, Plant cell walls and food quality, *Comp. Rev. Food Sci. Food Safety* 2 (2003) 101–119.
- [7] W.G. Glasser, W.E. Kaar, R.K. Jain, J.E. Sealey, Isolation options for non cellulosic heteropolysaccharides (HetPS), *Cellulose* 7 (2000) 299–317.
- [8] R.L. Whistler, Hemicelluloses, in: R.L. Whistler, J.N. BeMiller (Eds.), *Industrial Gums, Polysaccharides and their Derivatives*, (3rd ed.), Academic Press, San Diego, 1993, pp. 295–308.
- [9] Z.S. Cai, L. Paszner, Salt catalyzed wood bonding with hemicellulose, *Holzforschung* 42 (1988) 11–20.
- [10] C.M. Buchanan, N.L. Buchanan, J.S. Debenham, P. Gatenholm, M. Jacobsson, M.C. Shelton, T.L. Waterson, M.D. Wood, Preparation and characterization of arabinoxylan esters and arabinoxylan ester/cellulose ester polymer blends, *Carbohydr. Polym.* 52 (2003) 345–357.
- [11] A. Ebringerova, Z. Hromadkova, T. Heinze, Hemicellulose, *Adv. Polym. Sci.* 128 (2005) 1–67.
- [12] R. Boichicchio, F. Reicher, Are hemicelluloses from *Podocarpus lambertii* typical of gymnosperms? *Carbohydr. Polym.* 53 (2003) 127–136.
- [13] M.V.S.S.T.S. Rao, G. Muralikrishna, Non starch polysaccharides and bound phenolic acids from native and malted finger millet (*Ragi, Eleusine coracana*, Indaf 15), *Food Chem.* 72 (2001) 187–192.
- [14] A. Ebringerova, Z. Hromadkova, Xylans of industrial and biomedical importance, in: S.E. Harding (Ed.), *Biotechnology and Genetic Engineering Reviews*, Vol. 16, Intercept, England, 1999, pp. 325–346.
- [15] M. Colleoni Sirghie, B.D. Fulton, P.J. White, Structural features of water soluble (1,3) (1,4)  $\beta$  D glucans from high  $\beta$  glucan and traditional oat lines, *Carbohydr. Polym.* 54 (2003) 237–249.
- [16] M. Colleoni Sirghie, I.V. Kovalenko, J.L. Briggs, B. Fulton, P.J. White, Rheological and molecular properties of water soluble (1,3) (1,4)  $\beta$  D glucans from high  $\beta$  glucan and traditional oat lines, *Carbohydr. Polym.* 52 (2003) 439–447.
- [17] P.J. Wood, Relationships between solution properties of cereal  $\beta$  glucans and physiological effects a review, *Trends Food Sci. Technol.* 15 (2004) 313–320.
- [18] H.P. Ramesh, R.N. Tharanathan, Water extracted polysaccharides of selected cereals and influence of temperature on the extractability of polysaccharides in sorghum, *Food Chem.* 64 (1998) 345–350.
- [19] M.S. Izydorczyk, C.G. Biliaderis, Cereal arabinoxylans: advances in structure and physicochemical properties, *Carbohydr. Polym.* 28 (1995) 33–48.
- [20] G.B. Fincher, B.A. Stone, Cell walls and their components in cereal grain technology, in: Y. Pomeranz (Ed.), *Advances in Cereal Science and Technology*, Vol. 8, American Association of Cereal Chemists Inc., St Paul, 1986, pp. 207–295.
- [21] P.K. Kindel, S.Y. Liao, M.R. Liske, C.R. Olien, Arabinoxylans from rye and wheat seed that interact with ice, *Carbohydr. Res.* 187 (1989) 173–185.
- [22] J. Puls, J. Schuseil, Chemistry of hemicelluloses: relationship between hemicellulose structure and enzymes required for hydrolysis, in: M.P. Coughlan, G.P. Hazlewood (Eds.), *Hemicellulose and Hemicellulases*, Portland Press Research Monograph, 1993, pp. 1–27.
- [23] B. Xiao, X.F. Sun, R.C. Sun, Chemical, structural, and thermal characterizations of alkali soluble lingins and hemicelluloses, and cellulose from maize stems, rye straw, and rice straw, *Polym. Degrad. Stabil.* 74 (2001) 307–319.
- [24] S. Yoshida, I. Kusakabe, N. Matsuo, K. Shimizu, T. Yasui, K. Murakami, Structure of rice straw arabinoglucuronoxylan and specificity of *Streptomyces xylanase* toward the xylan, *Agric. Biol. Chem.* 54 (1990) 449–457.
- [25] R.C. Sun, X.F. Sun, G.Q. Liu, P. Fowler, J. Tomkinson, Structural and physicochemical characterization of hemicelluloses isolated by alkaline peroxide from barley straw, *Polym. Int.* 51 (2002) 117–124.
- [26] J.X. Sun, X.F. Sun, R.C. Sun, Y.Q. Su, Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses, *Carbohydr. Polym.* 56 (2004) 195–204.
- [27] A.G. Norman, The biological decomposition of plant materials, *Biochem. J.* 23 (1929) 1353–1366.
- [28] C.J.A. Vinkx, I. Stevens, H. Gruppen, P.J. Grobet, J.A. Delcour, Physicochemical and functional properties of rye nonstarch polysaccharides. VI. Variability in the structure of water unextractable arabinoxylans, *Cereal. Chem.* 72 (1995) 411–418.
- [29] M. Nilsson, L. Saulnier, R. Andersson, P.M. Aman, Water unextractable polysaccharides from three milling fractions of rye grain, *Carbohydr. Polym.* 30 (1996) 229–237.
- [30] M.E.F. Schooneveld Bergmans, A.M.C.P. Hopman, G. Beldman, A.G.J. Voragen, Extraction and partial characterization of

- feruloylated glucuronoarabinoxylans from wheat bran, *Carbohydr. Polym.* 35 (1998) 39 47.
- [31] L. Saulnier, C. Marot, E. Chanliaud, J.F. Thibault, Cell wall polysaccharide interactions in maize bran, *Carbohydr. Polym.* 26 (1995) 279 287.
  - [32] R.L. Whistler, Xylan, *Adv. Carbohydr. Chem.* 5 (1950) 269 290.
  - [33] T. Hayashi, Xyloglucans in the primary cell wall, *Ann. Rev. Plant Physiol.* 40 (1989) 139 168.
  - [34] G.O. Aspinall, Structural chemistry of the hemicelluloses, *Adv. Carbohydr. Chem.* 14 (1959) 429 468.
  - [35] O. Theander, Minor constituents of wheat flour: the pentosans, in: R.D. Hill, L. Munck (Eds.), *New Approaches to Research on Cereal Carbohydrates*, Elsevier, Amsterdam, 1985, pp. 217 230.
  - [36] I.M. Morrison, Changes in the hemicellulosic polysaccharides of ryegrass with increasing maturity, *Carbohydr. Res.* 36 (1974) 45 51.
  - [37] I.M. Morrison, The degradation and utilization of straw in the rumen, in: E. Grossbard (Ed.), *Straw Decay and its Effect on Disposal and Utilization*, Wiley, Chichester, 1979, pp. 237 245.
  - [38] G.O. Aspinall, Chemistry of cell wall polysaccharides, in: J. Presis (Ed.), *The Biochemistry of Plants*, Academic Press, New York, 1980, pp. 473 500.
  - [39] A.J. Buchala, C.G. Fraser, K.C.B. Wilkie, Acidic galactoarabinoxylan from the stem of *Avena sativa*, *Phytochemistry* 11 (1972) 2803 2814.
  - [40] Z. Benko, A. Andersson, Z. Szengyel, M. Gaspar, K. Reczey, H. Stalbrand, Heat extraction of corn fiber hemicellulose, *Appl. Biochem. BioTechnol.* 136 140 (2007) 253 266.
  - [41] J. Lenz, J. Schurz, J. Bauer, Thickening and gelation properties of hemicelluloses from the viscose process, *Papier* 38 (1984) 45 54.
  - [42] P. Paronen, M. Juslin, K. Kasanen, Comparison of xylan and some commercial materials as disintegrants in tablets, *Drug Dev. Ind. Pharm.* 11 (1985) 405 429.
  - [43] T. Persson, A.K. Nordin, G. Zacchi, A.S. Jonsson, Economic evaluation of isolation of hemicelluloses from process streams from thermomechanical pulping of spruce, *Appl. Biochem. BioTechnol.* 136 140 (2007) 741 752.
  - [44] A. Andersson, T. Persson, G. Zacchi, H. Stalbrand, A.S. Joensson, Comparison of diafiltration and size exclusion chromatography to recover hemicelluloses from process water from thermo mechanical pulping of spruce, *Appl. Biochem. BioTechnol.* 136 140 (2007) 971 984.
  - [45] K.K.Y. Wong, I.U.I. Tan, J.N. Saddler, Multiplicity of  $\beta$  1,4 xylanase in microorganisms: functions and applications, *Microbiol. Rev.* 52 (1988) 305 317.
  - [46] P. Biely, Microbial xylanolytic systems, *Trends BioTechnol.* 3 (1985) 286 290.
  - [47] O. Theander, P. Aman, Chemical composition of some Swedish cereal straws, *Swed. J. Agric. Res.* 8 (1978) 189 194.
  - [48] T.E. Timell, Isolation of galactoglucomannans from wood of gymnosperms, *Tappi J.* 44 (1961) 88 96.
  - [49] R.R. Selvendran, M.A. Oneill, Isolation and analysis of cell wall from plant material, *Methods Biochem. Anal.* 32 (1987) 25 153.
  - [50] J. Lundqvist, A. Jacobs, M. Palm, G. Zacchi, O. Dahlman, H. Stalbrand, Characterization of galactoglucomannan extracted from spruce (*Picea abies*) by heat fractionation at different conditions, *Carbohydr. Polym.* 51 (2003) 203 211.
  - [51] J.M. Lawther, R.C. Sun, W.B. Banks, Effects of extraction conditions and alkali type on yield and composition of wheat straw hemicellulose, *J. Appl. Polym. Sci.* 60 (1996) 1827 1837.
  - [52] J.M. Lawther, R.C. Sun, W.B. Banks, Effects of extraction conditions and alkali type on the yield and neutral sugar composition of wheat straw hemicellulose, *Chemistry and Processing of Wood and Plant Fibrous Materials (cellucon 94')*, Bangor, UK, Conference, 1996, pp. 313 344.
  - [53] R.C. Sun, J.M. Fang, P. Rowlands, J. Bolton, Physicochemical and thermal characterization of wheat straw hemicelluloses and celluloses, *J. Agric. Food Chem.* 46 (1998) 2804 2809.
  - [54] R.C. Sun, J.M. Lawther, W.B. Banks, Isolation and characterization of hemicellulose B and cellulose from pressure refined wheat straw, *Ind. Crop. Prod.* 7 (1998) 121 128.
  - [55] R.C. Sun, S. Hughes, Fractional extraction and physicochemical characterization of hemicelluloses and cellulose from sugarbeet pulp, *Carbohydr. Polym.* 36 (1998) 293 299.
  - [56] J.L. Ren, Z.C. Geng, C.F. Liu, F. Xu, J.X. Sun, R.C. Sun, Fractional isolation and structural characterization of hemicellulosic polymers from delignified and ultrasonic irradiated sugarcane bagasse, *E polymers* 67 (2006) 1 12.
  - [57] R.C. Sun, J.M. Fang, J. Tomkinson, Characterization and esterification of hemicelluloses from rye straw, *J. Agric. Food Chem.* 48 (2000) 1247 1252.
  - [58] D. Fengel, G. Wegener, *Wood Chemistry, Ultrastructure, Reactions*, Walter de Gruyter, Berlin, New York, 1989.
  - [59] D.S. Ruzene, D.P. Silva, A.A. Vicente, A.R. Goncalves, J.A. T, An alternative application to the portugures agro industrial residue: wheat straw, *Appl. Biochem. BioTechnol.* 147 (2008) 85 96.
  - [60] R.C. Sun, S. Hughes, Fractional isolation and physicochemical characterization of alkali soluble polysaccharides from sugar beet pulp, *Carbohydr. Polym.* 38 (1999) 273 281.
  - [61] R.C. Sun, X.F. Sun, Fractional and structural characterization of hemicelluloses isolated by alkali and alkaline peroxide from barley straw, *Carbohydr. Polym.* 49 (2002) 415 423.
  - [62] G.X. Pan, J.L. Bolton, G.J. Leary, Determination of ferulic and *p* coumaric acids in wheat straw and the amounts released by mild acid and alkaline peroxide treatment, *J. Agric. Food Chem.* 46 (1998) 5283 5288.
  - [63] J.M. Fang, R.C. Sun, J. Tomkinson, Isolation and characterization of hemicelluloses and cellulose from rye straw by alkaline peroxide extraction, *Cellulose* 7 (2000) 87 107.
  - [64] R.C. Sun, J. Tomkinson, Z.C. Geng, N.J. Wang, Comparative studies of hemicelluloses solubilized during the treatments of mainze stems with peroxy monosulfuric acid, peroxyformic acid, peracetic acid, and hydrogen peroxide. Part 1. Yield and chemical characterization, *Holzforchung* 54 (2000) 349 356.
  - [65] R.C. Sun, J. Tomkinson, Z.C. Geng, N.J. Wang, Comparative studies of hemicelluloses solubilized during the treatments of mainze stems with peroxy monosulfuric acid, peroxyformic acid, peracetic acid, and hydrogen peroxide. Part 2. Spectroscopic and thermal characterizations, *Holzforchung* 54 (2000) 492 496.
  - [66] R.C. Sun, J. Tomkinson, Essential guides for isolation and purification of polysaccharides, *Encyclopaedia Sep. Sci.* 6 (2000) 4568 4574.
  - [67] R.C. Sun, J. Tomkinson, P.L. Ma, S.F. Liang, Comparative study of hemicelluloses from rice straw by alkali and hydrogen peroxide treatments, *Carbohydr. Polym.* 42 (2000) 111 122.
  - [68] R.C. Sun, X.F. Sun, Fractional separation and structural characterization of lignins and hemicelluloses by a two stage treatment from rice straw, *Sep. Sci. Technol.* 37 (2002) 2433 2458.
  - [69] L.W. Doner, K.B. Hicks, Isolation of hemicellulose from corn fiber by alkaline hydrogen peroxide extraction, *Cereal Chem.* 74 (1997) 176 181.



- [70] Z. Hromadkova, J. Kovacikova, A. Ebringerova, Study of the classical and ultrasound assisted extraction of the corn cob xylan, *Ind. Crop. Prod.* 9 (1999) 101–109.
- [71] R.C. Sun, J. Tomkinson, F.C. Mao, X.F. Sun, Physicochemical characterization of lignins from rice straw by hydrogen peroxide treatment, *J. Appl. Polym. Sci.* 79 (2001) 719–732.
- [72] X.F. Sun, R.C. Sun, J. Tomkinson, M.S. Baird, Isolation and characterization of lignins, hemicelluloses, and celluloses from wheat straw by alkaline peroxide treatment, *Cell. Chem. Technol.* 37 (2003) 283–304.
- [73] R.C. Sun, J. Tomkinson, Y.X. Wang, B. Xiao, Physicochemical and structural characterization of hemicelluloses from wheat straw by alkaline peroxide extraction, *Polymer* 41 (1999) 2647–2656.
- [74] R.C. Sun, X.Y. Wang, X.F. Sun, J.X. Sun, Physicochemical and thermal characterisation of residual hemicelluloses isolated by TAED activated peroxide from ultrasonic irradiated and alkali organosolv pre treated wheat straw, *Polym. Degrad. Stabil.* 78 (2002) 295–303.
- [75] R.C. Sun, J. Tomkinson, Characterization of hemicelluloses isolated with tetraacetylenediamine activated peroxide from ultrasound irradiated and alkali pre treated wheat straw, *Eur. Polym. J.* 39 (2003) 751–759.
- [76] X.F. Sun, F. Xu, H. Zhao, R.C. Sun, P. Fowler, M.S. Baird, Physicochemical characterization of residual hemicelluloses isolated with cyanamide activated hydrogen peroxide from organosolv pre treated wheat straw, *Bioresource Technol.* 96 (2005) 1342–1349.
- [77] A.A. Shatalov, H. Pereira, Carbohydrate behavior of *Arundo donax* L. in ethanol alkali medium of variable composition during organosolv delignification, *Carbohydr. Polym.* 49 (2002) 331–336.
- [78] C. Vila, V. Santos, J.C. Parajo, Simulation of an organosolv pulping process: Generalized material balances and design calculations, *Ind. Eng. Chem. Res.* 42 (2003) 349–359.
- [79] C. Pascoal Neto, D. Evtuguin, A. Robert, Chemicals generated during oxygen organosolv pulping of wood, *J. Wood Chem. Technol.* 14 (1994) 383–402.
- [80] D.V. Evtuguin, I.P. Deineko, C. Pascoal Neto, Oxygen delignification in aqueous organic solvents media, *Cell. Chem. Technol.* 33 (1999) 103–123.
- [81] S. Abad, V. Santos, J.C. Parajo, Totally chlorine free bleaching of acetosolv pulps: a clean approach to dissolving pulp manufacture, *J. Chem. Technol. BioTechnol.* 76 (2001) 1117–1123.
- [82] F. Xu, C.F. Liu, Z.C. Geng, J.X. Sun, R.C. Sun, B.H. Hei, L. Lin, S.B. Wu, J. Je, Characterisation of degraded organosolv hemicelluloses from wheat straw, *Polym. Degrad. Stabil.* 91 (2006) 1880–1886.
- [83] F. Xu, J.X. Sun, C.F. Liu, R.C. Sun, Comparative study of alkali and acidic organic solvent soluble hemicellulosic polysaccharides from sugarcane bagasse, *Carbohydr. Res.* 341 (2006) 253–261.
- [84] X.F. Sun, R.C. Sun, P. Fowler, M.S. Baird, Extraction and characterization of original lignin and hemicelluloses from wheat straw, *J. Agric. Food Chem.* 53 (2005) 860–870.
- [85] A. Ebringerova, Z. Hromadkova, V. Hribalova, Structure and mitogenic activities of corn cob heteroxylans, *Int. J. Biol. Macromol.* 17 (1995) 327–331.
- [86] Z. Hromadkova, A. Ebringerova, Ultrasonic extraction of plant materials investigation of hemicellulose release from buckwheat hulls, *Ultrason. SonoChem.* 10 (2003) 127–133.
- [87] A. Ebringerova, Z. Hromadkova, J. Alföldi, V. Hribalova, The immunologically active xylan from ultrasound treated corn cobs: extractability, structure and properties, *Carbohydr. Polym.* 37 (1998) 231–239.
- [88] R.C. Sun, J. Tomkinson, Characterization of hemicelluloses isolated with tetraacetylenediamine activated peroxide from ultrasound irradiated and alkali pre treated wheat straw, *Eur. Polym. J.* 39 (2003) 751–759.
- [89] R.C. Sun, J. Tomkinson, Characterization of hemicelluloses obtained by classical and ultrasonically assisted extractions from wheat straw, *Carbohydr. Polym.* 50 (2002) 263–271.
- [90] J.X. Sun, R.C. Sun, X.F. Sun, Y.Q. Su, Fractional and physicochemical characterization of hemicelluloses from ultrasonic irradiated sugarcane bagasse, *Carbohydr. Res.* 339 (2004) 291–300.
- [91] R.C. Sun, X.F. Sun, X.H. Ma, Effect of ultrasound on the structural and physicochemical properties of organosolv soluble hemicelluloses from wheat straw, *Ultrason. SonoChem.* 9 (2002) 95–101.
- [92] S. N'Diaye, L. Rigal, P. Laroque, P.E. Vidal, Extraction of hemicelluloses from poplar, *Populus tremuloides*, using an extruder type twin screw reactor: a feasibility study, *Bioresource Technol.* 57 (1996) 61–67.
- [93] P. Marechal, J. Jorda, P.Y. Pontalier, L. Rigal, Twin screw extrusion and ultrafiltration for xylan production from wheat straw and bran, in: P. Gatenholm, M. Tenkanen (Eds.), *Hemicellulose: Science and Technology*, ACS symposium Series 864, Washington, DC, 2004, pp. 38–51.
- [94] M. Ibrahim, W.G. Glasser, Steam assisted biomass fractionation III: a quantitative evaluation of the “clean fractionation” concept, *Bioresource Technol.* 70 (1999) 181–192.
- [95] K. Shimizu, K. Sudo, H. Ono, M. Ishihara, T. Fujii, S. Hishiyama, Integrated process for total utilization of wood components by steam explosion pretreatment, *Biomass Bioenerg.* 14 (1998) 195–203.
- [96] X.F. Sun, F. Xu, R.C. Sun, Z.C. Geng, P. Fowler, M.S. Baird, Characteristics of degraded hemicellulosic polymers obtained from steam exploded wheat straw, *Carbohydr. Polym.* 60 (2005) 15–26.
- [97] H.Z. Chen, F.B. Sun, Steam explosion microwave coupling methods for separating components of plant straw, *Faming Zhuanli Shenqing Gongkai Shuomingshu* (2008), CN 101148830.
- [98] H.Z. Chen, L.Y. Liu, Unpolluted fractionation of wheat straw by steam explosion and ethanol extraction, *Bioresource Technol.* 98 (2007) 666–676.
- [99] R. Datar, J. Huang, P.C. Maness, A. Mohagheghi, S. Czernik, E. Chornet, Hydrogen production from the fermentation of corn stover biomass pretreated with a steam explosion process, *Int. J. Hydrogen Energ.* 32 (2007) 932–939.
- [100] K. Ohgren, A. Rudolf, M. Galbe, G. Zacchi, Fuel ethanol production from steam pretreated corn stover using SSF at higher dry matter content, *Biomass Bioenerg.* 30 (2006) 863–869.
- [101] F.A. Agblevor, M.M. Ibrahim, W.K. Ei Zawawy, Coupled acid and enzyme mediated production of microcrystalline cellulose from corn cob and cotton gin waste, *Cellulose* 14 (2007) 247–256.
- [102] M. Linde, M. Galbe, G. Zachhi, Simultaneous saccharification and fermentation of steam pretreated barley straw at low enzyme loadings and low yeast concentration, *Enzyme Microb. Technol.* 40 (2007) 1100–1107.
- [103] S.Y. Jin, H.Z. Chen, Fractionation of fibrous fraction from steam exploded rice straw, *Process Biochem.* 42 (2007) 188–192.
- [104] P. Luo, Z. Liu, G.S. Wang, Study of acid catalyzed steam explosion pretreatment for enzymatic hydrolysis of wheat straw, *Chem. Ind. forest Prod.* 26 (2006) 105–109.
- [105] M. Linde, E.L. Jakobsson, M. Galbe, G. Zacchi, Steam pretreatment of dilute  $H_2SO_4$  impregnated wheat straw and SSF with low yeast



- and enzyme loadings for bioethanol production, *Biomass Bioenerg.* 32 (2008) 326–332.
- [106] H. Chen, Y. Han, J. Xu, Simultaneous saccharification and fermentation of steam exploded wheat straw with alkaline peroxide, *Process Biochem.* 43 (2008) 1462–1466.
- [107] M.H. Thomsen, A. Thygesen, A.B. Thomsen, Hydrothermal treatment of wheat straw at pilot plant scale using a three step reactor system aiming at high hemicellulose recovery, high celluloses digestibility and low lignin hydrolysis, *Bioresource Technol.* 99 (2008) 4221–4228.
- [108] R.C. Sun, J.M. Lawther, W.B. Banks, Isolation and physicochemical characterization of xylose rich pectic polysaccharides from wheat straw, *Int. J. Polym. A.* 4 (1998) 345–356.
- [109] S. Zhu, Y. Wu, Z. Yu, X. Zhang, C.W. Wang, F.Q. Yu, S.W. Jin, Production of ethanol from microwave assisted alkali pretreated wheat straw, *Process Biochem.* 41 (2006) 869–873.
- [110] G. Garrote, H. Domínguez, J.C. Parajo, Kinetic modeling of corncob autohydrolysis, *Process Biochem.* 36 (2001) 571–578.
- [111] G. Garrote, H. Domínguez, J.C. Parajo, Autohydrolysis of corncob: study of non isothermal operation for xylooligosaccharides production, *J. Food Eng.* 52 (2002) 211–218.
- [112] J.C. Parajo, G. Garrote, J.M. Cruz, H. Domínguez, Production of xylooligosaccharides by autohydrolysis of lignocellulosic materials, *Trends Food Sci. Technol.* 15 (2004) 115–120.
- [113] M.J. Vazquez, G. Garrote, J.L. Alonso, H. Domínguez, J.C. Parajo, Enhancing the potential of oligosaccharides from corncob autohydrolysis as prebiotic food ingredients, *Ind. Crop. Prod.* 24 (2006) 152–159.
- [114] S. Makishima, M. Mizuno, N. Sato, K. Shinji, M. Suzuki, K. Nozaki, F. Takahashi, T. Kanda, Y. Amano, Development of continuous flow type hydrothermal reactor for hemicellulose fraction recovery from corn cob, *Bioresource Technol.* 100 (2009) 2842–2848.
- [115] H. Krawczyk, T. Persson, A. Andersson, A.S. Jonsson, Isolation of hemicelluloses from barley husks, *Food Bioprod. Process.* 86 (2008) 31–36.
- [116] A.A. Roos, T. Persson, H. Krawczyk, G. Zacchi, H. Stalbrand, Extraction of water soluble hemicelluloses from barley husks, *Bioresource Technol.* 100 (2009) 763–769.
- [117] J.S.G. Reid, K.C.B. Wilkie, Polysaccharides of the oat plant in relationship to plant growth, *Phytochemistry* 13 (1969) 2045–2051.
- [118] R.J. Vietor, S.A.G.F. Angelino, A.G.J. Voragen, Structure feature of arabinoxylans from barley and malt cell wall material, *J. Cereal Sci.* 15 (1992) 213–222.
- [119] H. Gruppen, R.J. Hamer, A.G.J. Voragen, Water unextractable cell wall material from wheat flour. 2. Fractionation of alkali extracted polymers and comparison with water extractable arabinoxylans, *J. Cereal Sci.* 16 (1992) 53–67.
- [120] R.A. Hoffmann, M. Roza, J. Maat, J.P. Kamerling, J.F.G. Vliegthart, Structural characteristics of the cold water soluble arabinoxylans from the white flour of the soft wheat variety Kadet, *Carbohydr. Polym.* 15 (1991) 415–430.
- [121] E. Haimer, M. Wendland, A. Potthast, T. Rosenau, F. Liebner, Precipitation of hemicelluloses from DMSO/water mixtures using carbon dioxide as an antisolvent, *J. Nanomater.* 2008 (2008) 1–5.
- [122] A. Ebringerova, Z. Hromadkova, E. Petrakova, M. Hricovini, Structural features of a water soluble L arabino D xylan from rye bran, *Carbohydr. Res.* 198 (1990) 57–66.
- [123] S. Bengtsson, P. Aman, Isolation and chemical characterization of water soluble arabinoxylans in rye grain, *Carbohydr. Polym.* 12 (1990) 267–277.
- [124] M.S. Izydorczyk, C.G. Biliaderis, Influence of structure on the physicochemical properties of wheat arabinoxylan, *Carbohydr. Polym.* 17 (1991) 237–247.
- [125] I. Gabriell, P. Gatenholm, W.G. Glasser, R.K. Jain, L. Kenne, Separation, characterization and hydrogel formation of hemicellulose from aspen wood, *Carbohydr. Polym.* 43 (2000) 367–374.
- [126] T. Imamura, T. Watanabe, M. Kuwahara, T. Koshijima, Ester linkages between lignin and glucuronic acid in lignincarbohydrate complexes from *Fagus crenata*, *Phytochemistry* 37 (1994) 165–173.
- [127] R.C. Sun, X.F. Sun, J.L. Wen, Fractional and structural characterization of lignins isolated by alkali and alkaline peroxide from barley straw, *J. Agric. Food Chem.* 49 (2001) 5322–5330.
- [128] T. Heinze, T. Liebert, A. Koschella, Analysis of polysaccharide structures, in: H.G. Barth, H. Pasch (Eds.), *Springer Laboratory Manuals in Polymer Science*, Springer Science+Business Media, 2006, pp. 15–39.
- [129] A. Teleman, J. Lundqvist, F. Tjerneld, H. Stalbrand, O. Dahlman, Characterization of acetylated 4-O-methylglucuronoxylan isolated from aspen employing  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy, *Carbohydr. Res.* 329 (2000) 807–815.
- [130] R.A. Hoffmann, T. Geijtenbeek, J.P. Kamerling, J.F.G. Vliegthart,  $^1\text{H}$  NMR study of enzymically generated wheat endosperm arabinoxylan oligosaccharides: structures of hepta to tetradeca saccharides containing two or three branched xylose residues, *Carbohydr. Res.* 223 (1992) 19–44.
- [131] H. Gruppen, R.A. Hoffmann, F.M. Kormelink, A.G.J. Voragen, J.P. Kamerling, J.F.G. Vliegthart, Characterization by proton NMR spectroscopy of enzymically derived oligosaccharides from alkali extractable wheat flour arabinoxylan, *Carbohydr. Res.* 233 (1992) 45–64.
- [132] D.J. Kiemle, A.J. Stipanovic, K.E. Mayo, Proton NMR methods in the compositional characterization of polysaccharides, in: P. Gatenholm, M. Tenkanen (Eds.), *Hemicelluloses: Science and Technology*, ACS Symposium Series 864, American Chemical Society, Washington, DC, 2004, pp. 122–139.
- [133] S.J. Shin, N.S. Cho, Conversion factors for carbohydrate analysis by hydrolysis and  $^1\text{H}$  NMR spectroscopy, *Cellulose* 15 (2008) 255–260.
- [134] Y. Copur, D. Kiemle, A. Stipanovic, J. Koskinen, M. Makkonen,  $^1\text{H}$  NMR spectroscopic determination of carbohydrates and yield in pine and maple pulps, *Pap. Puu Pap. Tim.* 3 (2003) 158–162.
- [135] M. Sutapa, L. Patrice, L.B. Corinne, D. Azeddine, R. Bimalendu, Structural characterisation of hemicellulosic polysaccharides from *Benincasa hispida* using specific enzyme hydrolysis, ion exchange chromatography and MALDI TOF mass spectroscopy, *Carbohydr. Polym.* 59 (2005) 231–238.
- [136] R. Bimalendu, L.B. Corinne, L. Catherine, C. Eric, D. Azeddine, L. Patrice, Structural investigation of hemicellulosic polysaccharides from *Argania spinosa*: characterization of a novel xyloglucan motif, *Carbohydr. Res.* 339 (2004) 201–208.
- [137] L. Olsson, H.R. Soerensen, B.P. Dam, H. Christensen, K.M. Krogh, A.S. Meyer, Separate and simultaneous enzymatic hydrolysis and fermentation of wheat hemicellulose with recombinant xylose utilizing *saccharomyces cerevisiae*, *Appl. Biochem. Biotechnol.* 129–132 (2006) 117–129.
- [138] S.H. Yoon, K. MacEwen, A.V. Heinigen, Hot water pre extraction from loblolly pine in an integrated forest products bio refinery, *Tappi J.* 7 (2008) 27–32.
- [139] H.R. Sorensen, A.S. Meyer, S. Pedersen, Enzymatic hydrolysis of water soluble wheat arabinoxylan. 1. Synergy between

- $\alpha$  L arabinofuranosidases, endo 1,4  $\beta$  xylanases, and  $\beta$  xylosidase activities, *Biotechnol. Bioeng.* 81 (2003) 726–731.
- [140] O.B. Dahlman, A. Jacobs, M. Nordstroem, Characterization of Hemicelluloses from Wood Employing Matrix Assisted laser Desorption/Ionization Time of Flight Mass Spectrometry, in: P. Gatenholm, M. Tenkanen (Eds.), *Hemicelluloses: Science and Technology*, ACS Symposium Series 864, American Chemical Society, Washington, DC, 2004, pp. 80–93.
- [141] A. Jacobs, O. Dahlman, Characterisation of the molar mass of hemicelluloses from wood and pulps employing SEC and MALDI TOF MS, *Biomacromolecules* 2 (2001) 894–905.
- [142] J.P. Joseleau, J. Comtat, K. Ruel, Chemical structure of xylans and their interaction in the plant cell walls, in: J. Visser (Ed.), *Xylans and Xylanases*, Elsevier, Amsterdam, 1992, pp. 1–15.
- [143] J.M. Lawther, R.C. Sun, W.B. Banks, Extraction, fractionation, and characterization of structural polysaccharides from wheat straw, *J. Agric. Food Chem.* 43 (1995) 667–675.
- [144] K.C.B. Wilkie, The hemicelluloses of grasses and cereals, *Adv. Carbohydr. Chem. Bi.* 36 (1979) 215–262.
- [145] A. Ebringerova, Z. Hromadkova, J. Alfoldi, G. Berth, Structural and solution properties of corn cob heteroxylans, *Carbohydr. Polym.* 19 (1992) 99–105.
- [146] T. Ishii, Structure and functions of feruloylated polysaccharides, *Plant Sci.* 127 (1997) 111–127.
- [147] F. Xu, R.C. Sun, J.X. Sun, C.F. Liu, B.H. He, J.S. Fan, Determination of cell wall ferulic and *p* coumaric acids in sugarcane bagasse, *Anal. Chim. Acta* 552 (2005) 207–217.
- [148] A. Kato, J. Azuma, T. Koshijima, Isolation and identification of a new ferulated tetrasaccharide from bagasse lignin carbohydrate complex containing phenolic acid, *Agric. Biol. Chem.* 51 (1987) 1691–1693.
- [149] Z.Q. Li, Hemicellulose, in: H.Y. Zhan, Z.Q. Li., Z.S. Cai (Eds.), *The Chemistry and Physical Property of Cellulose*, Science Press, Beijing, 2005, pp. 172–222.
- [150] S. Yoshida, A. Kuno, N. Saito, M. Aoyama, I. Kusakabe, Structure of xylan from culms of bamboo grass (*Sasa senanensis* Rehd.), *J. Wood Sci.* 44 (1998) 457–462.
- [151] I. Kusakabe, S. Ohgushi, T. Yasui, T. Kobayashi, Structures of the arabinoxylo oligosaccharides from the hydrolytic products of corn cob arabinoxylan by a xylanase from *Streptomyces*, *Agric. Biol. Chem.* 47 (1983) 2713–2723.
- [152] E. Maekawa, K. Kitao, Isolation and constitution of a xylan from bamboo, *Agric. Biol. Chem.* 37 (1973) 2073–2081.
- [153] M.D. Li, Chemical composition and structure property of bamboo hemicelluloses, *China Pulp Pap.* 3 (1990) 56–59.
- [154] T.E. Timell, Wood hemicelluloses, *Adv. Carbohydr. Chem.* 19 (1964) 247–302.
- [155] T.E. Timell, Wood hemicelluloses II, *Adv. Carbohydr. Chem.* 20 (1965) 409–483.
- [156] J.S.D. Bacon, A.H. Gordon, E.J. Morries, Acetyl groups in cell wall preparations from higher plants, *Biochem. J.* 149 (1975) 485–487.
- [157] H.O. Bouveng, P.J. Garegg, B. Lindberg, Position of the *O* acetyl groups in Birch xylan, *Chem. Ind.* 52 (1958) 1727–1727.
- [158] R.D. Hartley, Lignin carbohydrate linkages in plant cell walls. I. Carbohydrate esters of ferulic acid as components of cell walls of *Lolium multiflorum*, *Phytochemistry* 12 (1973) 661–665.
- [159] I. Mueller Harvey, R.D. Hartley, Linkage of *p* coumaroyl and feruloyl groups to cell wall polysaccharides of barley straw, *Carbohydr. Res.* 148 (1986) 71–85.
- [160] A. Scalbert, B. Monties, J.Y. Lallemand, E. Guittet, C. Rolando, Ether linkage between phenolic acids and lignin fractions from wheat straw, *Phytochemistry* 24 (1985) 1359–1362.
- [161] A. Scalbert, B. Monties, E. Guittet, J.Y. Lallemand, Comparison of wheat straw lignin preparations. I. Chemical and spectroscopic characterizations, *Holzforschung* 40 (1986) 119–127.
- [162] M. Ahmad, L. Khan, Spectroscopic studies of lignins from wheat straw and kai grass, *J. Chem. Soc. Pakistan.* 10 (1988) 299–302.
- [163] G. Almendros, A.T. Martínez, A.E. Gonzalez, F.J. Gonzalez Vila, R. Fründ, H.D. Lüdemann, CPMAS carbon 13 NMR study of lignin preparations from wheat straw transformed by five lignocellulose degrading fungi, *J. Agric. Food Chem.* 40 (1992) 1297–1302.
- [164] R.F. Helm, J. Ralph, Lignin hydroxycinnamyl model compounds related to forage cell wall structure. 1. Ether linked structures, *J. Agric. Food Chem.* 40 (1992) 2167–2175.
- [165] J.H. Grabber, R.D. Hatfield, J. Ralph, J. Zon, N. Amrhein, Ferulate crosslinking in cell walls isolated from maize cell suspensions, *Phytochemistry* 40 (1995) 1077–1082.
- [166] M.L. Fidalgo, M.C. Terron, A.T. Martinez, A.E. Gonzalez, F.J. Gonzalez vila, G.C. Galletti, Comparative study of fractions from alkaline extraction of wheat straw through chemical degradation, analytical pyrolysis, and spectroscopic techniques, *J. Agric. Food Chem.* 41 (1993) 1621–1626.
- [167] C. Crestini, D.S. Argyropoulos, Structural analysis of wheat straw lignin by quantitative <sup>31</sup>P and 2D NMR spectroscopy. The occurrence of ester bonds and  $\alpha$  O 4 Substructures, *J. Agric. Food Chem.* 45 (1997) 1212–1219.
- [168] K. Iiyama, T.B.T. Lam, Lignin in wheat internodes. Part 1: the reactivities of lignin units during alkaline nitrobenzene oxidation, *J. Sci. Food Agric.* 51 (1990) 481–491.
- [169] G. Jacquet, B. Pollet, C. Lapiere, F. Mhamdi, C. Rolando, New ether linked ferulic acid coniferyl alcohol dimers identified in grass straws, *J. Agric. Food Chem.* 43 (1995) 2746–2751.
- [170] T. Kondo, T. Ohshita, T. Kyuma, Comparison of characteristics of soluble lignins from untreated and ammonia treated wheat straw, *Anim. Feed Sci. Technol.* 39 (1992) 253–263.
- [171] J. Ralph, J.H. Grabber, R.D. Hatfield, Lignin ferulate crosslinks in grasses. 3. Lignin ferulate crosslinks in grasses: active incorporation of ferulate polysaccharide esters into ryegrass lignins, *Carbohydr. Res.* 275 (1995) 167–178.
- [172] T.B.T. Lam, K. Iiyama, B.A. Stone, Lignin in wheat internodes. Part 2: alkaline nitrobenzene oxidation by wheat straw lignin and its fractions, *J. Sci. Food Agric.* 51 (1990) 493–506.
- [173] T.B.T. Lam, K. Iiyama, B.A. Stone, Cinnamic acid bridges between cell wall polymers in wheat and phalaris internodes, *Phytochemistry* 31 (1992) 1179–1183.
- [174] M. Shimada, T. Fukuzuka, T. Higuchi, Ester linkages of *p* coumaric acid in bamboo and grass lignins, *Tappi* 54 (1971) 72–78.
- [175] R.C. Sun, J.M. Lawther, W.B. Banks, Fractional and structural characterization of ball milled and enzyme lignins from wheat straw, *J. Appl. Polym. Sci.* 68 (1998) 1633–1641.
- [176] J.G. Taylor, C.H. Haigler, Patterned secondary cell wall assembly in tracheary elements occurs in a self perpetuating cascade, *Acta Bot. Neerl.* 42 (1993) 153–163.
- [177] W. Bao, D.M. O'malley, R.R. Sederoff, Wood contains a cell wall structural protein, *Proc. Natl. Acad. Sci. U S A* 89 (1992) 6604–6608.
- [178] P. Wojtaszek, G.P. Bolwell, Secondary cell wall specific glycoprotein(s) from French bean hypocotyls, *Plant Physiol.* 108 (1995) 1001–1012.

- [179] A.C.E. Gregory, A.P. O'Connell, G.P. Bolwell, Xylans, *Biotechnol. Genet. Eng.* 15 (1998) 439 455.
- [180] N.J. Stacey, K. Roberts, N.C. Carpita, B. Wells, M.C. McCann, Dynamic changes in cell surface molecules are very early events in the differentiation of mesophyll cells from *Zinnia elegans* into tracheary elements, *Plant J.* 8 (1995) 891 906.
- [181] D.T.A. Lampion, L. Atona, S. Roerig, Galactosylserine in extension, *Biochem. J.* 133 (1973) 125 132.
- [182] F. Meuser, P. Suckow, Non starch polysaccharides, in: *Chemistry and Physics of baking*, J.M.V. Blanshard, P.J. Frazier, T. Galliard (Eds.), Royal Society of Chemistry, London, 1986, pp. 42 61.
- [183] D.T.A. Lampion, D.A. Miller, Hydroxyproline arabinosides in the plant kingdom, *Plant Physiol.* 48 (1971) 454 456.
- [184] R.C. Sun, J.M. Fang, A. Goodwin, J.M. Lawther, A.J. Bolton, Fractionation and characterization of ball milled and enzyme lignins from abaca fiber, *J. Sci. Food Agric.* 79 (1999) 1091 1098.
- [185] R.C. Sun, J.M. Lawther, W.B. Banks, Fractional isolation and physicochemical characterization of alkali soluble lignins from wheat straw, *Holzforschung* 51 (1997) 244 250.
- [186] R.C. Sun, J. Tomkinson, W. Zhu, S.Q. Wang, Delignification of maize stems by peroxymonosulfuric acid, peroxyformic acid, peracetic acid, and hydrogen peroxide. 1. Physicochemical and structural characterization of the solubilized lignins, *J. Agric. Food Chem.* 48 (2000) 1253 1262.
- [187] A. Chesson, A.H. Gordon, J.A. Lomax, Substituent groups linked by alkali labile bonds to arabinose and xylose residues of legume, grass and cereal straw cell walls and their fate during digestion by rumen microorganisms, *J. Sci. Food Agric.* 34 (1983) 1330 1340.
- [188] O. Eriksson, B.O. Lindgren, About the linkage between lignin and hemicelluloses in wood, *Sven. Papperstidn.* 80 (1977) 59 63.
- [189] T. Imamura, T. Watanabe, M. Kuwahara, T. Koshijima, Ester linkages between lignin and glucuronic acid in lignin carbohydrate complexes from *Fagus crenata*, *Phytochemistry* 37 (1994) 1165 1173.
- [190] J.M. Lawther, R.C. Sun, W.B. Banks, Extraction and comparative characterization of ball milled lignin (LM), enzyme lignin (LE) and alkali lignin (LA) from wheat straw, *Cell. Chem. Technol.* 30 (1996) 395 410.
- [191] R.C. Sun, J.M. Lawther, W.B. Banks, A tentative chemical structure of wheat straw lignin, *Ind. Crop. Prod.* 6 (1997) 1 8.
- [192] R.C. Sun, J.M. Lawther, W.B. Banks, Effects of extraction time and different alkalis on the composition of alkali soluble wheat straw lignins, *J. Agric. Food Chem.* 144 (1996) 3965 3970.
- [193] R.C. Sun, J.M. Lawther, W.B. Banks, Effects of pretreatment temperature and alkali concentration on the composition of alkali soluble lignins from wheat straw, *J. Appl. Polym. Sci.* 62 (1996) 1473 1481.
- [194] M. McNeil, P. Albersheim, L. Tainz, R.L. Jones, The structure of plant cell walls vii. barley aleurone cells, *Plant Physiol.* 55 (1975) 64 68.
- [195] J.P. Roubroeks, B. Saake, W. Glasser, P. Gatenholm, Contribution of the molecular architecture of 4 O methyl glucuronoxylan to its aggregation behavior in solution, in: P. Gatenholm, M. Tenkanen (Eds.), *Hemicelluloses: Science and Technology*, ACS Symposium Series 864, Washington, DC, 2004, pp. 167 183.
- [196] A. Ebringerova, Z. Hromadkova, W. Burchard, R. Dolega, W. Vorwerk, Solution properties of water insoluble rye bran Arabinoxylan, *Carbohydr. Polym.* 24 (1994) 161 169.
- [197] D.R. Picout, S.B. Ross Murphy, N. Errington, S.E. Harding, Pressure cell assisted solubilization of xyloglucans: tamarind seed polysaccharide and detarium gum, *Biomacromolecules* 4 (2003) 799 807.
- [198] Q. Wang, P.R. Ellis, S.B. Ross Murphy, W. Burchard, Solution characteristics of the xyloglucan extracted from *Detarium senega lense* Gmelin, *Carbohydr. Polym.* 33 (1997) 115 124.
- [199] A. Ebringerova, Z. Hromadkova, M. Kacurakova, M. Antal, Quaternized xylans: synthesis and structural characterization, *Carbohydr. Polym.* 24 (1994) 301 308.
- [200] J.D. Blake, G.N. Richards, Evidence for molecular aggregation in hemicelluloses, *Carbohydr. Res.* 18 (1971) 11 21.
- [201] R.C. Sun, J.M. Fang, J. Tomkinson, G.L. Jones, Acetylation of wheat straw hemicelluloses in *N,N* dimethylacetamide/LiCl solvent system, *Ind. Crop. Prod.* 10 (1999) 209 218.
- [202] J.M. Fang, R.C. Sun, J. Tomkinson, P. Fowler, Acetylation of wheat straw hemicelluloses B in a new non aqueous swelling system, *Carbohydr. Polym.* 41 (2000) 379 387.
- [203] J.M. Fang, R.C. Sun, P. Fowler, J. Tomkinson, C.A.S. Hill, Esterification of wheat straw hemicelluloses in the *N,N* Dimethyl formamide/Lithium chloride homogeneous system, *J. Appl. Polym. Sci.* 74 (1999) 2301 2311.
- [204] J.L. Ren, R.C. Sun, C.F. Liu, Z.N. Cao, W. Luo, Acetylation of wheat straw hemicelluloses in ionic liquid using iodine as a catalyst, *Carbohydr. Polym.* 70 (2007) 406 414.
- [205] K.A. Andrewartha, D.R. Phillips, B.A. Stone, Solution properties of wheat flour arabinoxylans and enzymically modified arabinoxylans, *Carbohydr. Res.* 77 (1979) 191 204.
- [206] U. Girhammar, M. Nakamura, B.M. Nair, Water soluble pentosans from wheat and rye chemical composition and some physical properties in solution, in: G.O. Philips, D.J. Wedlock, P.A. Williams (Eds.), *Gums and Stabilisers for the Food Industry*, Vol. 3, Elsevier, Amsterdam, 1986, pp. 123 134.
- [207] R. Dharmi, S.E. Harding, N.J. Elizabeth, A. Ebringerova, Hydrodynamic characterization of the molar mass and gross conformation of corn cob heteroxylan AGX, *Carbohydr. Polym.* 28 (1995) 113 119.
- [208] M.E.F. Schooneveld Bergmans, G. Beldman, A.G.J. Voragen, Structural features of (glucurono)arabinoxylans extracted from wheat bran by barium hydroxide, *J. Cereal Sci.* 29 (1999) 63 75.
- [209] E. Chanliaud, P. Roger, L. Saulnier, J.F. Thibault, Static and dynamic light scattering studies of heteroxylans from maize bran in aqueous solution, *Carbohydr. Polym.* 31 (1996) 41 46.
- [210] A. Ebringerova, Z. Hromadkova, The effect of ultrasound on the structure and properties of the water soluble corn hull heteroxylan, *Ultrason. SonoChem.* 4 (1997) 305 309.
- [211] A. Ebringerova, Z. Hromadkova, V. Hribalova, T.J. Mason, Effect of ultrasound on the immunogenic corn cob xylan, *Ultrason. SonoChem.* 4 (1997) 311 315.
- [212] M.S. Izydorczyk, L.J. Macri, A.W. MacGregor, Structure and physicochemical properties of barley non starch polysaccharides II. Alkali extractable  $\beta$  glucans and arabinoxylans, *Carbohydr. Polym.* 35 (1998) 259 269.
- [213] T.E. Eremeeva, T.O. Bykova, High performance size exclusion chromatography of wood hemicelluloses on a poly(2 hydroxyethyl methacrylate co ethylene dimethacrylate) column with sodium hydroxide solution as eluent, *J. Chromatogr.* 639 (1993) 159 164.
- [214] C.J.A. Vinkx, I. Van Haesendonck, S. Vanhamel, G. Cleemput, J. A. Delcour, Sephacryl S 500 HR gel filtration of water soluble arabinoxylan fractions from rye, *Prog. BioTechnol.* 7 (Xylans Xylanases), (1992) 387 390.
- [215] J. Lenz, E. Noggler, J. Leibetseder, Applicability of hemicellulose from beechwood pulp to increase the content of dietary fiber in baked goods and pastry products, *Nahrung* 30 (1986) 959 965.

- [216] J. Lenz, H. Wutzel, Effect of the addition of hemicelluloses on the flow properties of wheat flour dough, *Rheol. Acta* 23 (1984) 570–572.
- [217] Z. Hromadkova, A. Ebringerova, Rheological properties of beech wood xylan, *Papier* 45 (1991) 157–162.
- [218] Z. Hromadkova, A. Ebringerova, Rheological properties of beech wood xylan. II. Effect of the uronic acid side chains, *Papier* 47 (1993) 587–593.
- [219] U. Girhammar, B.M. Nair, Rheological properties of water soluble non starch polysaccharides from whole grain rye flour, *Food Hydrocolloid* 9 (1995) 133–140.
- [220] J. Michniewicz, C.G. Biliaderis, W. Bushuk, Water insoluble pentosans of wheat: composition and some physical properties, *Cereal Chem.* 67 (1990) 434–439.
- [221] A. Singh, V. Srivastava, M. Pandey, R. Sethi, R. Sanghi, Ipomoea turpethum seeds: a potential source of commercial gum, *Carbohydr. Polym.* 51 (2002) 357–359.
- [222] I.M. Sims, A.M. Gane, D. Dunstan, G.C. Allan, D.V. Boger, L.D. Melton, A. Bacic, Rheological properties of xyloglucans from different plant species, *Carbohydr. Polym.* 37 (1998) 61–69.
- [223] A. Lazaridou, C.G. Biliaderis, M. Michas, B.R. Steele, A comparative study on structure function relations of mixed linkage (1→3), (1→4) linear  $\beta$  D glucans, *Food Hydrocolloid* 18 (2004) 837–855.
- [224] C.L. Xu, S. Willfor, P. Holmlund, B. Holmbom, Rheological properties of water soluble spruce *O* acetyl galactoglucomannans, *Carbohydr. Polym.* 75 (2009) 498–504.
- [225] C.L. Xu, S. Willfor, B. Holmbom, Rheological properties of mixtures of spruce galactoglucomannans and konjac glucomannan or some other polysaccharides, *Bioresource* 3 (2008) 713–730.
- [226] A. Ebringerova, Z. Hromadkova, Flow properties of rye bran arabinoxylan dispersions, *Hydrocolloid* 6 (1992) 437–442.
- [227] A. Ng, R.N. Greenshields, K.W. Waldron, Oxidative cross linking of corn bran hemicellulose: formation of ferulic acid dehydromers, *Carbohydr. Res.* 303 (1997) 459–462.
- [228] R.C. Hoseney, J.M. Faubion, A mechanism for the oxidative gelation of wheat flour water soluble pentosans, *Cereal Chem.* 58 (1981) 421–424.
- [229] F. Meuser, P. Suckow, A. Abd Elgawad, Characterization of pentosan fractions from wheat. 2. Study of the gelling ability of pentosans and glycoproteins from the wheat varieties Diplomat and Maris Huntsman, *Veroeffentlichungen der Arbeitsgemeinschaft Getreideforschung* 183 (1981) 103–112.
- [230] A.J. Varma, J.F. Kennedy, P. Galgali, Synthetic polymers functionalized by carbohydrates: a review, *Carbohydr. Polym.* 56 (2004) 429–445.
- [231] M.E.F. Schooneveld Bergmans, Y.M. Van Dijk, G. Beldman, A.G.J. Voragen, Physicochemical characteristics of wheat bran glucuronoarabinoxylans, *Cereal Sci.* 29 (1999) 49–61.
- [232] D.K. Sarker, P.J. Wilde, D.C. Clark, Enhancement of protein foam stability by formation of wheat arabinoxylan protein crosslinks, *Cereal Chem.* 75 (1998) 493–499.
- [233] C.J.A. Vinkx, J.A. Delcour, Rye (*Secale cereale* L.) arabinoxylans: A critical review, *J. Cereal Sci.* 24 (1996) 1–14.
- [234] C.G. Biliaderis, M.S. Izydorczyk, O. Rattan, Effect of arabinoxylans on bread making quality of wheat flours, *Food Chem.* 53 (1995) 165–171.
- [235] I. Gabrieli, P. Gatenholm, Preparation and properties of hydrogels based on hemicellulose, *J. Appl. Polym. Sci.* 69 (1998) 1661–1667.
- [236] B.S. Kayserlioglu, U. Bakir, L. Yilmaz, N. Akkas, Use of xylan, an agricultural by product, in wheat gluten based biodegradable films: mechanical, solubility and water vapor transfer rate properties, *Bioresource Technol.* 87 (2003) 239–246.
- [237] D.A.I. Goring, Softening of lignin, hemicelluloses, and cellulose, *Pulp Paper Mag. Can.* 64 (1963) T517–T527.
- [238] A. Ebringerova, J. Hirsch, A. Kramar, R. Domansky, Thermal decomposition of hardwood hemicelluloses in the 80 to 200 deg. range. II. Changes in the chemical properties of native 4 *O* methylglucuroxylan, *Cell. Chem. Technol.* 8 (1974) 61–68.
- [239] J.M. van Hazendonk, E.J.M. Reinerink, P. de Waard, J.E.G. van Dam, Inter glycoside acetals. Part 3. Synthesis and structure determination of cyclic monobenzyldiene acetals of cyclodextrin derivatives bridging between two continuous D glucopyranosyl residues, *Carbohydr. Res.* 291 (1996) 53–62.
- [240] C. Devallencourt, J.M. Saiter, D. Capitaine, Characterization of recycled celluloses: thermogravimetry/Fourier transform infrared coupling and thermogravimetry investigations, *Polym. Degrad. Stabil.* 52 (1996) 327–334.
- [241] K. Van de Velde, P. Kiekens, Thermal degradation of flax: the determination of kinetic parameters with thermogravimetric analysis, *J. Appl. Polym. Sci.* 83 (2002) 2634–2644.
- [242] S.G. Lee, E.Y. An, J.B. Lee, J.C. Park, J.W. Shin, J.K. Kim, Enhanced cell affinity of poly(D,L lactic co glycolic acid) (50/50) by plasma treatment with  $\beta$  (1→3) (1→6) glucan, *Surf. Coat. Technol.* 201 (2007) 5128–5131.
- [243] Y.Q. Zhang, B.J. Xie, X. Gan, Advance in the applications of konjac glucomannan and its derivatives, *Carbohydr. Polym.* 60 (2005) 27–31.
- [244] T. Coviello, P. Matricardi, C. Marianecchi, F. Alhaique, Polysaccharide hydrogels for modified release formulations, *J. Control. Release* 119 (2007) 5–24.
- [245] R.L. Whistler, A.A. Bushway, P.P. Singh, W. Nakahara, R. Tokuzen, Noncytotoxic, antitumor polysaccharides, *Adv. Carbohydr. Chem. Bi.* 32 (1976) 235–375.
- [246] N.G. Asp, I. Björck, M. Nyman, Physiological effects of cereal dietary fiber, *Carbohydr. Polym.* 21 (1993) 183–187.
- [247] Z. Hromadkova, A. Ebringerova, Hemicelluloses for nutrition and health, *Chem. Listy (in Slovak)* 88 (1994) 591–603.
- [248] A. Chesson, Dietary fiber, in: A.M. Stephen (Ed.), *Food Polysaccharides and Their Applications*, Marcel Dekker, New York, 1995, pp. 547–576.
- [249] P.A. Baghurst, K.I. Baghurst, S.J. Record, Dietary fibre, non starch polysaccharides and resistant starch – a review, *Food Aust.* 48 (1996) S3–S35.
- [250] T. Kodama, K. Shiba, K. Tsuji, Suppressive effect of wheat bran hemicellulose on blood pressure in spontaneously hypertensive rats, *Nippon Eiyo Shokuryo Gakkaishi* 2 (1996) 101–105.
- [251] P. Ryden, J.A. Robertson, The effect of fiber source and fermentation on the apparent hydrophobic binding properties of wheat bran preparations for the mutagen 2 amino 3,8 dimethylimidazo[4,5 f] quinoxaline (MeIQx), *Carcinogenesis* 16 (1995) 209–216.
- [252] K. Angkanaporn, M. Choct, W.L. Bryden, E.F. Annison, G. Annison, Effects of wheat pentosans on endogenous amino acid losses in chickens, *J. Sci. Food Agric.* 66 (1994) 399–404.
- [253] G. Annison, P.J. Moughan, D.V. Thomas, Nutritive activity of soluble rice bran arabinoxylans in broiler diets, *Brit. Poultry Sci.* 36 (1995) 479–488.
- [254] A.K.A. Silva, E.L. da Silva, E.E.J. Oliveira, L.A.L. Soares, A.C. Medeiros, J.H. Araujo, I.B. Araujo, A.S. Carrico, E.S.T. Egitto, Synthesis and characterization of xylan coated magnetite microparticles, *Int. J. Pharm.* 334 (2007) 42–47.



- [255] S. Yllner, B. Enstrom, Adsorption of xylan on cellulose fibers during the sulfate cook. I, *Sven. Papperstidn.* 59 (1956) 229–232.
- [256] S. Yllner, B. Enstrom, The adsorption of xylan on cellulose fibers during the sulfate cook. II, *Sven. Papperstidn.* 60 (1957) 449–454.
- [257] J.A. Hansson, N. Hartler, Sorption of hemicelluloses on cellulose fibers. I. Sorption of xylans, *Sven. Papperstidn.* 72 (1969) 521–530.
- [258] M. Mitikka, R. Teeaar, M. Tenkanen, J. Laine, T. Vuorinen, Sorption of xylans on cellulose fibers, In 8th International Symposium on Wood Pulping Chemistry; Gummerus Kirjapaino Oy, Jyväskylä, Finland, 1995, pp. 231–236.
- [259] J.C. Roberts, A.S. El Karim, The behavior of surface adsorbed xylans during the beating of a bleached kraft pine pulp, *Cell. Chem. Technol.* 17 (1983) 379–386.
- [260] C. Schonberg, T. Oksanen, A. Suurnakki, H. Kettunen, J. Buchert, The importance of xylan for the strength properties of spruce kraft pulp fibres, *Holzforschung* 55 (2001) 639–644.
- [261] R.H. Marchessault, W.J. Settineri, W. Winter, Crystallization of xylan in the presence of cellulose, *Tappi* 50 (1967) 55–59.
- [262] F. Mora, K. Ruel, J. Comtat, J.P. Joseleau, Aspect of native and redeposited xylans at the surface of cellulose microfibrils, *Holzforschung* 40 (1986) 85–91.
- [263] L.R. Lynd, Overview and evaluation of fuel ethanol from cellulosic biomass: technology, economics, the environment, and policy, *Annu. Rev. Energ. Env.* 21 (1996) 403–465.
- [264] T.T. Teeri, H.III. Brumer, G. Daniel, P. Gatenholm, Biomimetic engineering of cellulose based materials, *Trends BioTechnol.* 25 (2007) 299–306.
- [265] A. Henriksson, P. Gatenholm, Controlled assembly of glucuronoxylans onto cellulose fibres, *Holzforschung* 55 (2001) 494–502.
- [266] Å. Henriksson, P. Gatenholm, Surface properties of CTMP fibers modified with xylans, *Cellulose* 9 (2002) 55–64.
- [267] A. Linder, R. Bergman, A. Bodin, P. Gatenholm, Mechanism of assembly of xylan onto cellulose surfaces, *Langmuir* 19 (2003) 5072–5077.
- [268] A. Linder, J.P. Roubroeks, P. Gatenholm, Effect of ozonation on assembly of xylans, *Holzforschung* 57 (2003) 496–502.
- [269] N.C. Carpita, M. Defernez, K. Findlay, B. Wells, D.A. Shoue, G. Catchpole, R.H. Wilson, M.C. McCann, Cell wall architecture of the elongating maize coleoptile, *Plant Physiol.* 127 (2001) 551–565.
- [270] T. Iwata, L. Indrarti, J.I. Azuma, Affinity of hemicellulose for cellulose produced by acetobacter xylinum, *Cellulose* 5 (1998) 215–228.
- [271] E.M. Kerr, S.C. Fry, Pre formed xyloglucans and xylans increase in molecular weight in three distinct compartments of a maize cell suspension culture, *Planta* 217 (2003) 327–339.
- [272] D. Reis, B. Vian, Helicoidal patterns in secondary cell walls and possible role of xylans in their construction, *CR. Biol.* 327 (2004) 785–790.
- [273] M.A. Kabel, H. van den Borne, J.P. Vincken, A.G.J. Voragen, H.A. Schols, Structural differences of xylans affect their interaction with celluloses, *Carbohydr. Polym.* 69 (2007) 94–105.
- [274] A. Esker, U. Becker, S. Jamin, S. Beppu, S. Renneckar, W. Glasser, Self assembly behavior of some co- and heteropolysaccharides related to hemicelluloses, in: P. Gatenholm, M. Tenkanen (Eds.), *Hemicelluloses: Science and Technology*, ACS Symposium Series 864, American Chemical Society, Washington, DC, 2004, pp. 198–219.
- [275] T. Kohnke, C. Pujolras, J.P. Roubroeks, P. Gatenholm, The effect of barley husk arabinoxylan adsorption on the properties of cellulose fibres, *Cellulose* 15 (2008) 537–546.
- [276] T. Hannuksela, B. Holmbom, Sorption of mannans to different fiber surfaces: an evolution of understanding, in: P. Gatenholm, M. Tenkanen (Eds.), *Hemicelluloses: Science and Technology*, ACS Symposium Series 864, American Chemical Society, Washington, DC, 2004, pp. 222–235.
- [277] V.A. Russo, E.F. Thode, Studies of a modified locust bean gum on a bleached sulfite pulp, *Tappi* 43 (1960) 209–218.
- [278] J.V. Robinson, Fiber bonding, in: J.P. Casey (Ed.), *Pulp and Paper Chemistry and Chemical Technology*, Vol. 2, Wiley, New York, 1980, pp. 915–961.
- [279] F. Mobarak, A.E. Ei Ashmany, Y. Fahmy, Hemicelluloses as additive in paper making. Part II. The role of added hemicelluloses and hemicellulose in situ on paper properties, *Cell. Chem. Technol.* 7 (1973) 323–325.
- [280] K.X. Hu, Study on the utilization of modified hemicelluloses from reed, *Hu Nan Papermaking* 2 (1998) 28–31.
- [281] J. Laine, P. Stenius, Effect of charge on the fibre and paper properties of bleached industrial kraft pulps, *Pap. Puu* 79 (1997) 257–266.
- [282] A. Suurnakki, T. Oksanen, H. Kettunen, J. Buchert, The effect of mannan on physical properties of ECF bleached softwood kraft fibre handsheets, *Nord. Pulp Pap. Res. J.* 18 (2003) 429–435.
- [283] T. Hannuksela, P. Fardim, B. Holmbom, Sorption of spruce *O* acetylated galactoglucomannans onto different pulp fibres, *Cellulose* 10 (2003) 317–324.
- [284] D.U. Lima, R.C. Oliveira, M.S. Buckeridge, Seed storage hemicelluloses as wet end additives in papermaking, *Carbohydr. Polym.* 52 (2003) 367–373.
- [285] S. Danielsson, M.E. Lindstrom, Influence of birch xylan adsorption during kraft cooking on softwood pulp strength, *Nord. Pulp Pap. Res. J.* 20 (2005) 436–441.
- [286] B.J. Lao, New usage of hemicellulose, *Heilong Pulp Pap.* 32 (2004) 17–18.
- [287] A. Naterova, J. Kucera, A. Ebringerova, Application of xylan in papermaking, *Vyskumne Prace z Odboru Papiera a Celulozy* 41 (1986) V17–V28, V23–V26, V30.
- [288] B. Saake, T. Busse, J. Puls, The effect of xylan adsorption on the properties of sulfite and kraft pulps, *Proceedings of the 13th ISWFPC*, Vol. 2, Auckland, New Zealand, 2005, pp. 141–146.
- [289] J.L. Ren, R.C. Sun, F. Peng, B.H. He, L. Lin, Application of cationic hemicelluloses as strengthening agent in OCC pulp, *China Pulp Pap.* 26 (2007) 14–16.
- [290] J.L. Ren, F. Peng, R.C. Sun, J.F. Kennedy, Influence of hemicellulosic derivatives on the sulfate kraft pulp strength, *Carbohydr. Polym.* 75 (2009) 338–342.
- [291] J.L. Ren, F. Peng, R.C. Sun, The effect of hemicellulosic derivatives on the strength properties of old corrugated container pulp fibres, *J. Biobased Mater Bio.* 3 (2009) 62–68.
- [292] F. Ramirez, J. Puls, V. Zuniga, B. Saake, Sorption of corn bob and oat spelt arabinoxylan onto softwood kraft pulp, *Holzforschung* 62 (2008) 329–337.
- [293] A. Paananen, M. Osterberg, M. Rutland, T. Tammellin, T. Saarinen, K. Tappura, P. Stenius, Interaction between cellulose and xylan: an atomic force microscope and quartz crystal microbalance study, in: P. Gatenholm, M. Tenkanen (Eds.), *Hemicelluloses: Science and Technology*, ACS Symposium Series 864, American Chemical Society, Washington, DC, 2004, pp. 269–290.
- [294] J.X. Sun, F.C. Mao, X.F. Sun, R.C. Sun, Comparative study of hemicelluloses isolated with alkaline peroxide from lignocellulosic materials, *J. Wood Chem. Technol.* 24 (2005) 239–262.



- [295] T. Jeoh, C.I. Ishizawa, M.F. Davis, M.E. Himmel, W.S. Adney, D.K. Johnson, Cellulase digestibility of pretreated biomass is limited by cellulose accessibility, *Biotechnol. Bioeng.* 98 (2007) 112–122.
- [296] K. Pakkala, M. Kontturi, A. Kallioinen, O. Myllymaki, J. Uusitalo, M. Siika aho, N. von Weymarn, Production of bio ethanol from barley straw and reed canary grass, 15th European Biomass Conference and Exhibition, Berlin Germany, 2007, pp. 154–157.
- [297] Y. Nakamura, T. Sawada, E. Inoue, Enhanced ethanol production from enzymatically treated steam exploded rice straw using extractive fermentation, *J. Chem. Technol. BioTechnol.* 76 (2001) 879–884.
- [298] M.G. Jackson, Review articles: the alkali treatment of straws, *Anim. Feed Sci. Technol.* 2 (1977) 105–130.
- [299] R.R. Spencer, D.E. Akin, Rumen microbial degradation of potassium hydroxide treated coastal bermuda grass leaf blades examined by electron microscopy, *J. Anim. Sci.* 51 (1980) 1189–1196.
- [300] R.W. Detroy, R.L. Cunningham, R.J. Bothast, M.O. Bagby, A. Herman, Bioconversion of wheat straw cellulose/hemicellulose to ethanol by *Saccharomyces uvarum* and *Pachysolen tannophilus*, *Biotechnol. Bioeng.* 24 (2004) 1105–1113.
- [301] J.N. Nigam, Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*, *J. BioTechnol.* 87 (2001) 17–27.
- [302] N.J. Cao, M.S. Krishnan, J.X. Du, C.S. Gong, N.W.Y. HO, Z.D. Chen, G.T. Tsao, Ethanol production from corn cob pretreated by the ammonia steeping process using genetically engineered yeast, *Biotechnol. Lett.* 18 (1996) 1013–1018.
- [303] B.C. Saha, L.B. Iten, M.A. Cotta, Y.V. Wu, Dilute acid pretreatment, enzymatic saccharification, and fermentation of wheat straw to ethanol, *Process Biochem.* 40 (2005) 3693–3700.
- [304] M.H. Thomsen, A. Thygesen, H. Joergensen, J. Larsen, B.H. Christensen, A.B. Thomsen, Preliminary results on optimization of pilot scale pretreatment of wheat straw used in coproduction of bioethanol and electricity, *Appl. Biochem. BioTechnol.* 129 (2006) 448–460.
- [305] A. Pesson, I.M. Mancilha, S. Sato, Acid hydrolysis of hemicellulose from sugarcane bagasse, *Braz. J. Chem. Eng.* 14 (1997) 291–297.
- [306] R. Brunecky, T.B. Vinzant, S.E. Porter, B.S. Donohoe, D.K. Johnson, M.E. Himmel, Redistribution of xylan in maize cell walls during dilute acid pretreatment, *Biotechnol. Bioeng.* 102 (2009) 1537–1543.
- [307] P.F. Gao, D.D. Fan, Y.E. Luo, X.X. Ma, P. Ma, J.F. Hui, C.H. Zhu, Efficient and comprehensive utilization of hemicellulose in the corn stover, *Chinese J. Chem. Eng.* 17 (2009) 350–354.
- [308] B.C. Saha, M.A. Cotta, Enzymatic hydrolysis and fermentation of lime pretreated wheat straw to ethanol, *J. Chem. Technol. BioTechnol.* 82 (2007) 913–919.
- [309] V.S. Chang, B. Burr, M.T. Holtzapple, Lime pretreatment of wheat straw, *Appl. Biochem. BioTechnol.* 63 (1997) 3–19.
- [310] W.E. Karr, M.T. Holtzaple, Using lime pretreatment to facilitate the enzyme hydrolysis of corn stover, *Biomass Bioenerg.* 18 (2000) 189–199.
- [311] S. Kim, M.T. Holtzapple, Lime pretreatment and enzymatic hydrolysis of corn stover, *Bioresource Technol.* 96 (2005) 994–2006.
- [312] B.C. Saha, M.A. Cotta, Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw, *Biotechnol. Progr.* 22 (2006) 449–453.
- [313] K.K. Cheng, J.A. Zhang, W.X. Ping, J.P. Ge, Y.J. Zhou, H.Z. Ling, J.M. Xu, Sugarcane bagasse mild alkaline/oxidative pretreatment for ethanol production by alkaline recycle process, *Appl. Biochem. BioTechnol.* 151 (2008) 43–50.
- [314] B.C. Saha, R.J. Bothast, Enzymes in lignocellulosic biomass conversion, in: B.C. Saha, J. Woodward (Eds.), *Fuels and chemical from biomass*, American Chemical Society, Washington, D.C., 1997, pp. 46–56.
- [315] P.T. Larrson, Interaction between cellulose I and hemicelluloses studied by spectral fitting of CP/MAS  $^{13}\text{C}$  NMR spectra, in: P. Gatenholm, M. Tenkanen (Eds.), *Hemicellulose: Science and Technology*, ACS Symposium Series 864, American Chemical Society, Washington, DC, 2004, pp. 254–268.
- [316] I. Duchesne, E.L. Hult, U. Molin, G. Daniel, T. Iversen, H. Lennholm, The influence of hemicellulose on fibril aggregation of kraft pulp fibres as revealed by FE SEM and CP/MAS  $^{13}\text{C}$  NMR, *Cellulose* 8 (2001) 103–111.
- [317] K. Wickholm, P.T. Larsson, T. Iversen, Assignment of noncrystalline forms in celluloses I by CP/MAS  $^{13}\text{C}$  NMR spectroscopy, *Carbohydr. Res.* 312 (1998) 123–129.
- [318] I. Duchesne, G. Daniel, Chinages in surface ultrastructure of Norway spruce fibres during kraft pulping visualization by field emission SEM, *Nord. Pulp Pap. Res. J.* 15 (2000) 54–61.
- [319] J.F. Kennedy, A.J. Griffiths, K. Philp, D.L. Stevenson, O. Kambanis, C.J. Gray, Characteristics and distributions of ester groups in propylene glycol alginates, *Carbohydr. Polym.* 11 (1989) 1–22.
- [320] C.L. Smart, R.L. Whistler, Films from hemicellulose acetates, *Science* 110 (1949) 713–714.
- [321] M.S. Lindblad, E. Ranucci, A.C. Albertsson, Biodegradable polymers from renewable sources, New hemicellulose based hydrogels, *Macromol. Rapid Commun.* 22 (2001) 962–967.
- [322] E. Hagglund, B. Lindberg, J. McPherson, Dimethylsulphoxide, a solvent for hemicelluloses, *Acta Chem. Scand.* 10 (1956) 1160–1164.
- [323] B. Focher, A. Marzetti, A. Naggi, G. Torri, Sulfopropylated hemicellulose: synthesis and NMR characterization, *Makromol. Chem.* 190 (1989) 129–138.
- [324] J.F. Carson, W.D. Maclay, Esters of lima bean pod and corn cob hemicelluloses, *J. Am. Chem. Soc.* 70 (1948) 293–295.
- [325] S. Thiebaud, M.E. Borredon, Analysis of the liquid fraction after esterification of sawdust with octanoyl chloride production of esterified hemicelluloses, *Bioresource Technol.* 63 (1998) 139–145.
- [326] C. Moine, V. Gloaguen, J.M. Gloaguen, R. Granet, P. Krausz, Chemical valorization of forest and agricultural by products. Obtention chemical characteristics, and mechanical behavior of a novel family of hydrophobic films, *J. Environ. Sci. Health B* 39 (2004) 627–640.
- [327] R.C. Sun, J.M. Fang, J. Tomkinson, Stearoylation of hemicelluloses from wheat straw, *Polym. Degrad. Stabil.* 67 (2000) 345–353.
- [328] J.M. Fang, R.C. Sun, P. Fowler, J. Tomkinson, C.A.S. Hill, Esterification of wheat straw hemicelluloses in the *N,N* dimethyl formamide/lithium chloride homogenous system, *J. Appl. Polym. Sci.* 79 (2000) 719–732.
- [329] F. Peng, J.L. Ren, B. Peng, F. Xu, R.C. Sun, J.X. Sun, Rapid homogeneous lauroylation of wheat straw hemicelluloses under mild conditions, *Carbohydr. Res.* 343 (2008) 2956–2962.
- [330] X.F. Sun, R.C. Sun, J.X. Sun, Oleoylation of sugarcane bagasse hemicelluloses using *N* bromosuccinimide as a catalyst, *J. Sci. Food Agric.* 84 (2004) 800–810.
- [331] R.L. Shogren, A. Biswas, Preparation of water soluble and water swellable starch acetates using microwave heating, *Carbohydr. Polym.* 64 (2006) 16–21.
- [332] J.L. Ren, F. Xu, R.C. Sun, B. Peng, J.X. Sun, Studies of the lauroylation of wheat straw hemicelluloses under heating, *J. Agric. Food Chem.* 56 (2008) 1251–1258.

- [333] F. Xu, J.X. Jiang, R.C. Sun, D. She, B. Peng, J.X. Sun, J.F. Kennedy, Rapid esterification of wheat straw hemicelluloses induced by microwave irradiation, *Carbohydr. Polym.* 73 (2008) 612–620.
- [334] F. Peng, J.L. Ren, X.F. Sun, F. Xu, R.C. Sun, B. Peng, J.X. Sun, Rapid phthaloylation and succinylation of hemicelluloses by microwave irradiation, *E polymers* 108 (2008) 1–11.
- [335] X.F. Sun, R.C. Sun, J.X. Sun, Acetylation of sugarcane bagasse hemicelluloses under mild reaction conditions by using nbs as a catalyst, *J. Appl. Polym. Sci.* 92 (2004) 53–61.
- [336] R.C. Sun, X.F. Sun, F.Y. Zhang, Succinylation of wheat straw hemicelluloses in *N,N* dimethylformamide/lithium chloride system, *Polym. Int.* 50 (2001) 803–811.
- [337] R.C. Sun, S.T. Min, X.F. Sun, Preparation and characterization of wheat straw hemicellulosic succinates, *Int. J. Polym. Anal. Chem.* 7 (2002) 130–140.
- [338] X.F. Sun, R.C. Sun, J. Tomkinson, M.S. Baird, Preparation of sugarcane bagasse hemicellulosic succinates using NBS as a catalyst, *Carbohydr. Polym.* 53 (2003) 483–495.
- [339] R.C. Sun, X.F. Sun, X. Bing, Succinylation of wheat straw hemicelluloses with a low degree of substitution in aqueous systems, *J. Appl. Polym. Sci.* 83 (2002) 757–766.
- [340] S. Barthel, T. Heinze, Acylation and carbanilation of cellulose in ionic liquids, *Green Chem.* 8 (2006) 301–306.
- [341] T. Heinze, K. Schwikal, S. Barthel, Ionic liquids as reaction medium in cellulose functionalization, *Macromol. Biosci.* 5 (2005) 520–525.
- [342] C.M.G.C. Renard, M.C. Jarvis, Acetylation and methylation of homogalacturonans. 1. Optimization of the reaction and characterization of the products, *Carbohydr. Polym.* 39 (1999) 201–207.
- [343] J.Z. Li, T. Furuno, S. Katoh, T. Uehara, Chemical modification of wood by anhydrides without solvents or catalysts, *J. Wood Sci.* 46 (2000) 215–221.
- [344] R.M. Rowell, A.M. Tillman, R. Simonson, A simplified procedure for the acetylation of hardwood and softwood flakes for flakeboard production, *J. Wood Chem. Technol.* 6 (1986) 427–428.
- [345] K.A. Connors, K.S. Albert, Determination of hydroxy compounds by 4 dimethylaminopyridine catalyzed acetylation, *J. Pharm. Sci.* 62 (1973) 845–846.
- [346] C.A.S. Hill, N.S. Cetin, Z. Ozmen, Potential catalysts for the acetylation of wood, *Holzforschung* 54 (2000) 269–272.
- [347] G. Hofl, W. Steglich, H. Vorbruggen, 4 Dialkylaminopyridines as acylation catalysts. 5. Simple method for the esterification of carboxylic acids, *Angew. Chem.* 90 (1978) 556–557.
- [348] G. Hofler, W. Steglich, H. Vorbruggen, New synthetic methods. 25. 4 dialkylaminopyridines as acylation catalysts. 4. 4 dialkylamino pyridines as highly active acylation catalysts, *Angew. Chem.* 90 (1978) 602–615.
- [349] C.L. McCormick, P.A. Callais, Derivatization of cellulose in lithium chloride and *N,N* dimethylacetamide solutions, *Polymer* 28 (1987) 2317–2323.
- [350] S.L. Williamson, C.L. McCormick, Cellulose derivatives synthesized via isocyanate and activated ester pathways in homogeneous solutions of lithium chloride *N,N* dimethylacetamide, *J. Macromol. Sci. Pure A* 35 (1988) 1915–1927.
- [351] R.C. Sun, X.F. Sun, J. Tomkinson, Hemicelluloses and their derivatives, in: P. Gatenholm, M. Tenkanen (Eds.), *Hemicelluloses: Science and Technology*, ACS Symposium Series 864, American Chemical Society, Washington, DC, 2004, pp. 2–18.
- [352] B. Philipp, I. Nehls, W. Wagenknecht, M. Schnabelrauch, Schnabelrauch, <sup>13</sup>C NMR spectroscopic study of the homogeneous sulphation of cellulose and xylan in the N<sub>2</sub>O<sub>4</sub> DMF system, *Carbohydr. Res.* 164 (1987) 107–116.
- [353] M.M.D. Hussein, W.A. Helmy, H.M. Salem, Biological activities of some galactomannans and their sulfated derivatives, *Phytochemistry* 48 (1998) 479–484.
- [354] K. Takechi, K.I. Furuhashi, Synthesis and nucleophilic substitution of tosylated konjac glucomannan, *Sen'i Gakkaishi* 55 (1999) 315–322.
- [355] R.G. Schweiger, Nitrite esters of polyhydroxy polymers, *J. Org. Chem.* 41 (1976) 90–93.
- [356] I. Croon, A. Donetzhuber, Xanthation and dexanthation of some hemicellulose fractions, *Tappi J.* 46 (1963) 648–651.
- [357] V. Haack, T. Heinze, G. Oelmeyer, W.M. Kulicke, Starch derivatives of high degree of functionalization, 8a synthesis and Xoculation behavior of cationic starch polyelectrolytes, *Macromol. Mater. Eng.* 287 (2002) 495–502.
- [358] L.M. Zhang, Preparation and anti clay swelling ability of new water soluble cellulose derivatives containing quaternary ammonium groups, *J. Appl. Polym. Sci.* 79 (2001) 1416–1422.
- [359] M.M. Thanou, A.F. Kotze, T. Scharringhausen, H.L. Luessen, A.G. de Boer, J.C. Verhoef, H.E. Junginger, Effect of degree of quaternization of N trimethyl chitosan chloride for enhanced transport of hydrophilic compounds across intestinal Caco 2 cell monolayers, *J. Control. Release* 64 (2000) 15–25.
- [360] T. Heinze, A. Koschella, A. Ebringerova, Chemical functionalization of xylan: a short review, in: P. Gatenholm, M. Tenkanen (Eds.), *Hemicelluloses: Science and Technology*, ACS Symposium Series 864, American Chemical Society, Washington, DC, 2004, pp. 312–325.
- [361] I. Simkovic, J. Mlynar, J. Alfoldi, M.M. Micko, New aspects in cationization of lignocellulose materials. XI. Modification of bagasse with quaternary ammonium groups, *Holzforschung* 44 (1990) 113–116.
- [362] I. Simkovic, J. mlynar, J. Alfoldi, Modification of corn cob meal with quaternary ammonium groups, *Carbohydr. Polym.* 17 (1992) 285–288.
- [363] M. Antal, A. Ebringerova, I. Simkovic, New aspects in cationization of lignocellulose materials II. Distribution of functional groups in lignin, hemicellulose, and cellulose components, *J. Appl. Polym. Sci.* 29 (1984) 643–650.
- [364] M. Antal, A. Ebringerova, M.M. Micko, Cationic hemicelluloses from aspen wood flour and their use in paper production, *Papier* 45 (1991) 232–235.
- [365] A. Ebringerova, Z. Hromadkova, Substituent distribution in cationic xylan derivatives, *Angew. Makromol. Chem.* 24 (1996) 97–104.
- [366] A. Ebringerova, A. Belicova, L. Ebringer, Antimicrobial activity of quaternized heteroxylans, *World J. Microb. Biot.* 10 (1994) 640–644.
- [367] A. Ebringerova, Z. Hromadkova, M. Kacurakova, M. Antal, Quaternized D xylans: synthesis, structure and properties, Book of abstracts on the VIIth European Carbohydrate Symposium, Cracow, 1993, pp. D003.
- [368] J.L. Ren, R.C. Sun, C.F. Liu, L. Lin, B.H. He, Synthesis and characterization of novel cationic SCB hemicelluloses with a low degree of substitution, *Carbohydr. Polym.* 67 (2007) 347–357.
- [369] J.L. Ren, F. Peng, R.C. Sun, C.F. Liu, Z.N. Cao, W. Luo, J.N. Tang, Synthesis of cationic hemicellulosic derivatives with a low degree of substitution in dimethyl sulfoxide media, *J. Appl. Polym. Sci.* 109 (2008) 2711–2717.
- [370] J.L. Ren, R.C. Sun, C.F. Liu, Z.Y. Chao, W. Luo, Two step preparation and thermal characterization of cationic 2 hydroxypropyltrimethylammonium chloride hemicellulose polymers from sugarcane bagasse, *Polym. Degrad. Stabil.* 91 (2006) 2579–2587.

- [371] J.L. Ren, C.F. Liu, R.C. Sun, D. She, C.F. Liu, Preparation and characterization of sugarcane bagasse hemicellulosic derivatives containing quaternary ammonium groups in various media, *E polymers* 061 (2007) 1–9.
- [372] J.L. Ren, R.C. Sun, C.F. Liu, Etherification of hemicelluloses from sugarcane bagasse, *J. Appl. Polym. Sci.* 105 (2007) 3301–3308.
- [373] T. Heinze, New ionic polymers by cellulose functionalization, *Macromol. Chem. Phys.* 199 (1998) 2341–2364.
- [374] F. Cheng, G.F. Li, J.X. Feng, J.W. Zhang, Characteristics of carboxymethyl cellulose synthesized in two phase medium  $C_6H_6$   $C_2H_5OH$ . 1. Distribution of substituent groups in the anhydroglucose unit, *J. Appl. Polym. Sci.* 61 (1996) 1831–1838.
- [375] L.M. Kooijmann, K.J. Ganzeveld, R.M. Manurung, H.J. Heeres, Experimental studies on the carboxymethylation of arrowroot starch in isopropanol water media, *Starch Starke* 55 (2003) 495–503.
- [376] D.A. Silva, R.C.M. Paula, J.P.A. Feitosa, A.C.F. Brito, J.S. Maciel, H.C.B. Paula, Carboxymethylation of cashew tree exudate poly saccharide, *Carbohydr. Polym.* 58 (2004) 163–171.
- [377] J.L. Ren, R.C. Sun, F. Peng, Carboxymethylation of hemicelluloses isolated from sugarcane bagasse, *Polym. Degrad. Stabil.* 93 (2008) 786–793.
- [378] M.S. Dudkin, E.A. Kogan, S.I. Grinshpun, Synthesis and properties of carboxymethyl ethers of xylan, *Latvijas PSR Zinatnu Akademijas Vestis, Kimijas Serija* 5 (1994) 633–637.
- [379] J. Schomorak, G.A. Adams, The preparation and properties of carboxymethyl xylan, *Tappi J.* 40 (1957) 378–383.
- [380] K. Petzold, K. Schwikal, T. Heinze, Carboxymethyl synthesis and detailed structure characterization, *Carbohydr. Polym.* 64 (2006) 292–298.
- [381] K. Petzold, K. Schwikal, W. Gunther, T. Heinze, Carboxymethyl xylan control of properties by synthesis, *Macromol. Symp.* 232 (2006) 27–36.
- [382] S. Saghir, M.S. Iabal, M.A. Hussain, A. Koschella, T. Heinze, Structure characterization and carboxymethylation of arabinoxylan isolated from Ispaghula (*Plantago ovata*) seed husk, *Carbohydr. Polym.* 74 (2008) 309–317.
- [383] J.L. Ren, F. Peng, R.C. Sun, Preparation of hemicellulosic derivatives with bifunctional groups in different media, *J. Agric. Food Chem.* 56 (2008) 11209–11216.
- [384] J.L. Ren, F. Peng, R.C. Sun, Preparation and characterization of hemicellulosic derivatives containing carbamoyl ethyl and carboxy ethyl groups, *Carbohydr. Res.* 343 (2008) 2776–2782.
- [385] J.L. Ren, F. Peng, R.C. Sun, X.L. Zhang, Preparation and characterization of carbamoyl ethyl hemicelluloses, *E polymers* 3 (2009) 1–11.
- [386] M. Zinbo, T.E. Timell, The degree of branching of hardwood xylans, *Sven. Papperstidn.* 68 (1965) 647–662.
- [387] J.M. Fang, P. Fowler, J. Tomkinson, C.A.S. Hill, Preparation and characterization of methylated hemicelluloses from wheat straw, *Carbohydr. Polym.* 47 (2002) 285–293.
- [388] R.K. Jain, M. Sjostedt, W.G. Glasser, Thermoplastic xylan derivatives with propylene oxide, *Cellulose* 7 (2000) 319–336.
- [389] W.G. Glasser, R.K. Jain, M.A. Sjostedt, Thermoplastic pentosan rich polysaccharides from biomass, *Biotechnol. Adv.* 14 (1996) 605–605.
- [390] A. Ebringerova, Z. Novotna, M. Kacurakova, E. Machova, Chemical modification of beechwood xylan with p carboxybenzyl bromide, *J. Appl. Polym. Sci.* 62 (1996) 1043–1047.
- [391] A. Ebringerova, Z. Hromadkova, A. Malovikova, V. Sasinkova, J. Hirsch, I. Srokova, Structure and properties of water soluble p carboxybenzyl polysaccharide derivatives, *J. Appl. Polym. Sci.* 78 (2000) 1191–1199.
- [392] A. Ebringerova, J. Alfoldi, Z. Hromadkova, G.M. Pavlov, S.E. Harding, Water soluble p carboxybenzylated beechwood 4 O methylglucuronoxylan: structural features and properties, *Carbohydr. Polym.* 42 (2000) 123–131.
- [393] A.R. Mahdavian, S. Khoei, Trends in modification of polymers, in: R.K. Bregg (Ed.), *Horizons in Polymer Research*, Nova Science Publishers, 2005, pp. 83–114.
- [394] M.S. Lindblad, A.C. Albertsson, in: S. Dumitriu (Ed.), *Polysaccharides: Structural diversity and functional versatility*, Marcel Dekker, New York, 2005, pp. 491–508.
- [395] B. Raenby, H. Sundstroem, Graft copolymerization onto native cellulosic fibers using manganese ( $3^+$ ) initiation, *Eur. Polym. J.* 19 (1983) 1067–1070.
- [396] J.C. Arthur Jr., Cellulose graft copolymers, *J. Macromol. Sci. Part A: Pure Appl. Chem.* 4 (1970) 1057–1065.
- [397] G.F. Fanta, R.C. Burr, W.M. Doane, Graft polymerization of acrylonitrile and methyl acrylate onto hemicellulose, *J. Appl. Polym. Sci.* 27 (1982) 4239–4250.
- [398] N.A. El Shinnawy, S.F. El Kalyoubi, Graft copolymerization of acrylonitrile onto hemicellulose using ceric ammonium nitrate, *J. Appl. Polym. Sci.* 30 (1985) 2171–2178.
- [399] A.A.A. Soliman, Graft copolymerization of acrylonitrile onto hemicellulose and its effect on paper sheets, *J. Sci. Ind. Res. India.* 56 (1977) 545–552.
- [400] C. Peroval, F. Debeaufort, A.M. Seuvre, P. Cayot, B. Chevet, D. Despre, A. Voilley, Modified arabinoxylan based films Grafting of functional acrylates by oxygen plasma and electron beam irradiation, *J. Membr. Sci.* 233 (2004) 129–139.
- [401] M.F. Ishak, T. Painter, Formation of inter residue hemiacetals during the oxidation of polysaccharides by periodate ion, *Acta Chem. Scand.* 25 (1971) 3875–3877.
- [402] B. Augustinsson, O. Larm, E. Scholander, Oxidation of methyl  $\alpha$  and  $\beta$  D xylopyranoside and a xylan with bromine in the presence of borate, *Carbohydr. Res.* 152 (1986) 305–309.
- [403] R.L. Taylor, H.E. Conrad, Stoichiometric depolymerization of polyuronides and glycosaminoglycans to monosaccharides following reduction of their carbodiimide activated carboxyl groups, *Biochemistry* 11 (1972) 1383–1388.
- [404] S. Matsumura, S. Maeda, S. Yoshikawa, Molecular design of biodegradable poly(carboxylic acid), *Polymer materials science and engineering*, 62, American Chemical Society, Division of Polymeric Materials Science and Engineering, Washington, DC, 1990, p. 984.
- [405] S. Matsumura, M. Nishioka, S. Yoshikawa, Molecular design of biodegradable functional polymers, 2. Poly(carboxylic acid) containing xylopyranosediyl groups in the backbone, *Makromol. Chem.* 191 (1990) 1269–1274.
- [406] S. Matsumura, M. Nishioka, S. Yoshikawa, Enzymatically degradable poly(carboxylic acid) derived from polysaccharide, *Makromol. Chem. Rapid Commun.* 12 (1991) 89–94.
- [407] J.R. Jerez, B. Matsuhira, C.C. Urzua, Chemical modification of the xylan from *Palmaria decipiens*, *Carbohydr. Polym.* 32 (1997) 155–159.
- [408] N.P. Barroso, J. Costamagna, B. Matsuhira, M. Wilagran, El xilano de *palmaria decipiens*: modification quimica y formacion de un complejo de Cu(II), *Bol. Soc. Chil. Quim.* 42 (1997) 301–306.
- [409] E. Fredon, R. Granet, R. Zerrouki, P. Krausz, L. Saulnier, J.F. Thibault, J. Rosier, C. Petit, Hydrophobic films from maize bran hemicelluloses, *Carbohydr. Polym.* 49 (2002) 1–12.

- [410] S.I. Mussatto, I.C. Roberto, Kinetic behaviour of *Candida guilliermondii* yeast during xylitol production from highly concentrated hydrolysate, *Process Biochem.* 39 (2004) 1433–1439.
- [411] L. Canilha, S.J.B. Almeida, A.I.N. Solenzal, Eucalyptus hydrolysate detoxification with activated charcoal adsorption or ion exchanger resins for xylitol production, *Process Biochem.* 39 (2004) 1909–1912.
- [412] W.C. Liaw, C.S. Chen, W.S. Chang, K.P. Chen, Xylitol production from rice straw hemicellulose hydrolyzate by polyacrylic hydrogel thin films with immobilized *Candida subtropicalis* WF79, *J. Biosci. Bioeng.* 105 (2008) 97–105.
- [413] L. Farooq, I.R. Mohammad, Production of ethanol and xylitol from corn cobs by yeasts, *Bioresource Technol.* 77 (2001) 57–63.
- [414] K.K. Cheng, J.A. Zhang, H.Z. Ling, W.X. Ping, W. Huang, J.P. Ge, J.M. Xu, Optimization of pH and acetic acid concentration for bioconversion of hemicellulose from corncobs to xylitol by *Candida tropicalis*, *Biochem. Eng. J.* 43 (2009) 203–207.
- [415] I.M. Solange, D. Giuliano, C.R. Ines, Influence of the toxic compounds present in brewer's spent grain hemicellulosic hydrolysate on xylose to xylitol bioconversion by *Candida guilliermondii*, *Process Biochem.* 40 (2005) 3801–3806.
- [416] W. Carvalho, J.C. Santos, L. Canilha, S.S. Silva, P. Perego, A. Converti, Xylitol production from sugarcane bagasse hydrolysate: metabolic behaviour of *Candida guilliermondii* cells entrapped in Ca alginate, *Biochem. Eng. J.* 25 (2005) 25–31.
- [417] I.M. Mancilha, M.N. Karim, Evaluation of ion exchange resins for removal of inhibitory compounds from corn stover hydrolyzate for xylitol fermentation, *Biotechnol. Progr.* 19 (2003) 1837–1841.
- [418] R.C. Sun, J.M. Fang, A. Goodwin, J.M. Lawther, A.J. Bolton, Isolation and characterization of polysaccharides from abaca fiber, *J. Agric. Food Chem.* 46 (1998) 2817–2822.
- [419] M. Gaspar, T. Juhasz, Z. Szengyel, K. Reczey, Fractionation and utilization of corn fibre carbohydrates, *Process Biochem.* 40 (2005) 1183–1188.
- [420] C.H. Unlu, E. Gunister, O. Atici, Synthesis and characterization of NaMt biocomposites with corn cob xylan in aqueous media, *Carbohydr. Polym.* 76 (2009) 585–592.
- [421] M. Hashi, T. Takeshita, Studies on antitumor activity of wood hemicelluloses. II. The host mediated antitumor effect of 4-O-methylglucuronoxylan, *Agric. Biol. Chem.* 43 (1979) 961–967.
- [422] A. Ebringerova, A. Kardosova, Z. Hromadkova, A. Malovikova, V. Hribalova, Immunomodulatory activity of acidic xyans in relation to their structural and molecular properties, *Int. J. Biol. Macromol.* 30 (2002) 1–6.
- [423] H. Wanger, A. Proksch, I. Riess Mauter, A. Vollmar, S. Odenthal, S. Stuppner, H. Stuppner, Immunstimulierend wirkende polysaccharide (heteroglykane) aus höheren pflanzen, *Arzneimittel forschung* 35 (1985) 1069–1075.
- [424] A. Proksch, H. Wanger, Structural analysis of a 4-O-methylglucuronoarabinoxylan with immuno stimulating activity from *Echinacea purpurea*, *Phytochemistry* 26 (1987) 1989–1993.
- [425] E. Fuller, S. Sosa, A. Tubaro, G. Franz, R.D. Loggia, Anti inflammatory activity of Chamomilla polysaccharides, *Planta Med.* 59 (1993) 666–667.
- [426] H. Yamada, T. Nagai, T.G. Cyong, Y. Otsuka, Relationship between chemical structure and anti complementary activity of plant polysaccharides, *Carbohydr. Res.* 144 (1985) 101–111.
- [427] A.B. Samuelson, B.S. Paulsen, J.K. Wold, H. Otsuka, H. Yamada, T. Espevik, Isolation and partial characterization of biologically active polysaccharides from *Plantago major L.*, *Phytother. Res.* 9 (1995) 211–218.



# Cellulose

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## 5.1 OCCURRENCE

At present, cellulose is the most abundant polymer available worldwide with an estimated annual natural production of  $1.5 \times 10^{12}$  tons and considered as an almost inexhaustible source of raw materials [1, 2]. As a raw material of an enormous underutilized energy resource for the production of paper, panel products, chemicals, and other industrial products, cellulose has received much attention around all over the world.

In general, nonwood materials such as cereal straws, grasses, and sugarcane bagasse (SCB) are composed of single fiber cells that are only about 0.5–3.0 mm in length, whereas bast fibers such as flax can have single cells as long as 77 mm [3]. Cellulose is the main constituent of cell wall in lignocellulosic plant, and its content depends on the plant species, growing environment, position, growth, and maturity. Generally, cellulose content in lignocellulosic plant is 23–53% on a dry-weight basis, less than that in cotton, which is almost made of pure fibrous cellulose [4]. In most straw species, approximately 35–45% of the dry substance is cellulose. On the dry matter, the cellulose content of wheat straw, rice straw, rye straw, barley straw, oat straw, bagasse, maize stems, and corn cobs is 38.6, 36.5, 37.9, 34.8, 38.5, 39.2, and 43.2%, respectively, which is similar to the cellulose content of esparto and oil palm fiber (35.8 and 40.2%, respectively); however, the cellulose content of these straws is less than that of abaca fiber, 60.4%, and more than that of sugar beet pulp, 18.4% [5].

Plant cell walls provide strength and support for plants, limit the size and shape of cells, and act as a barrier to potential pathogens. In straw cell walls, cellulose exists as microfibrils of indefinite length and varying degrees of crystallinity and is embedded in a gel matrix composed of hemicelluloses, lignins, and other carbohydrate polymers [6, 7]. Cellulose microfibrils are of great importance in straw tissues and make the major contribution to the mechanical strength of the cell walls and act as the

framework of cell wall [8]. The morphology and orientation of cellulose in cell walls of straws have been reported. Yu et al. [7] reported the morphology and orientation of cellulose in the vessels of vascular bundles of wheat straw. In the vascular bundles, cellulose acts as the framework, and cellulose chains are high in orientation. In the thickening part of the vessels, cellulose exists in the form of cellulose crystalline lamellae but not cellulose microfibrils. The crystalline lamellae are perpendicular to the tangential direction of annular rings and incline clockwise with an angle of about  $30^{\circ}$ – $40^{\circ}$  to the tangential direction of the spiral line in the spiral vessels. They also proposed a model of the arrangement of cellulose chains in the vascular bundles, as shown in Fig. 5.1.

Cellulose is a linear homopolymer composed of D-glucopyranose units linked by  $\beta$ -1,4-glycosidic bonds ( $C_{6n}H_{10n+2}O_{5n+1}$  ( $n$  = degree of polymerization of glucose)), as shown in Fig. 5.2.

As the main component of cell wall, cellulose is predominantly located in the secondary wall. In the primary cell wall, cellulose consists of roughly 6000 glucose

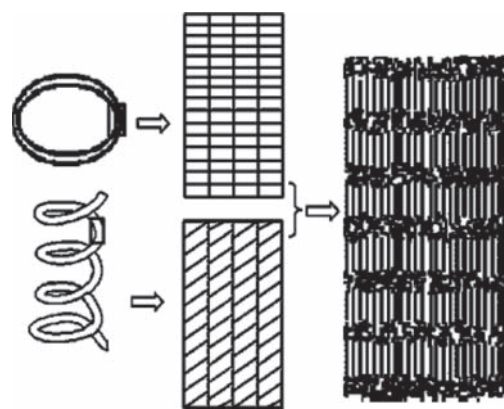


FIGURE 5.1 A schematic model of cellulose molecules in the annular and spiral vessels [7].



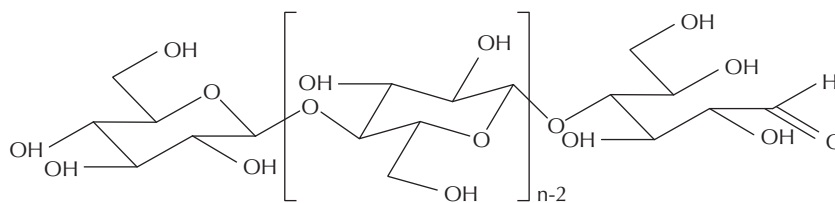


FIGURE 5.2 Chemical structure of cellulose chain.

units, and in the secondary wall, the number increases to 13 000–16 000 units. A cellulose microfibril with a diameter of 20–30 nm contains about 2000 molecules. Reddy et al. [9] reported that wheat straw cellulose fibers have coarser (wider width) single cells than cotton, linen, and kenaf. The breaking tenacity (force at break) of wheat straw fibers is similar to that of kenaf but lower than that of cotton and linen, their percentage of breaking elongation is similar to that of linen and kenaf but lower than that of cotton, and their Young's modulus is similar to cotton but lower than that of linen and kenaf. This indicated that the cellulose fiber has the potential as natural fiber for textile, composite, and other fibrous applications. The cellulose samples isolated from both dejuiced ryegrass leaves and the untreated leaves were found to have much shorter fibers (0.35–0.49 mm) than those of cereal straws, bagasse, or wood [10].

## 5.2 ISOLATION

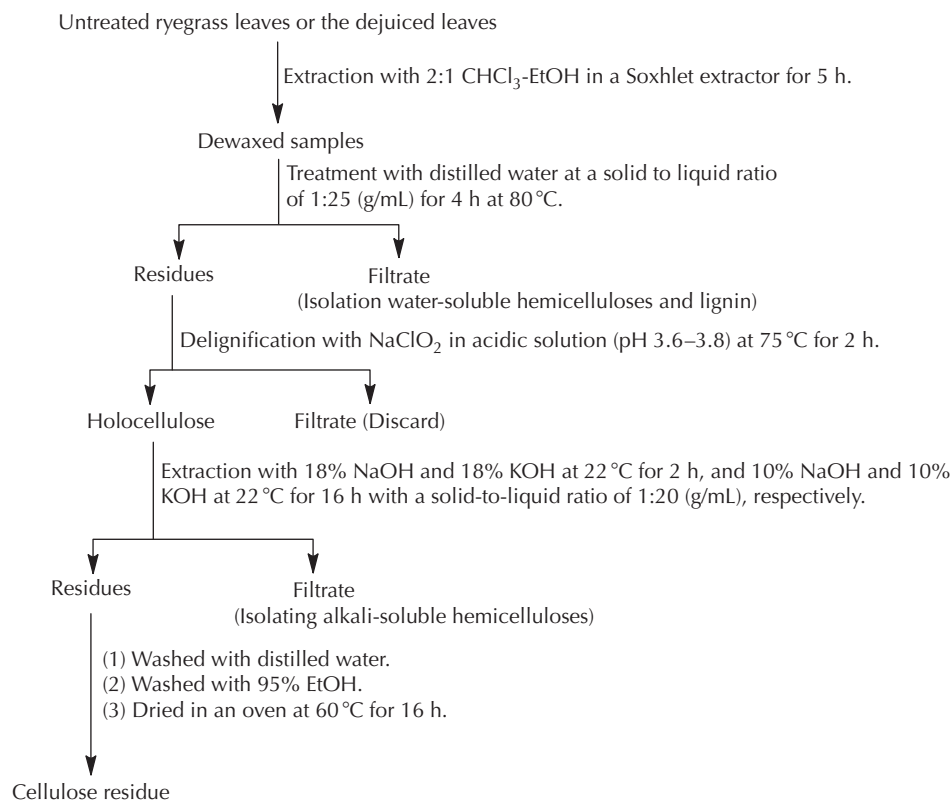
In the lignocellulosic materials, cellulose is embedded in a gel matrix composed of hemicelluloses, lignins, and other carbohydrate polymers [6, 7]. The isolation of highly pure cellulose has been the subject of extensive studies for many years because of the complexity of cell wall structure [11, 12]. The combination of the chemical and the mechanical treatments is necessary for the dissolution of lignins, hemicelluloses, and other noncellulosic substances. A protocol based on acidified sodium chlorite is frequently applied to delignify woody materials as an initial step in the isolation of cellulose [13]. Alkali extraction to dissolve hemicelluloses before or after delignification is the common method [14]. It is well known that treatment of the lignocellulosic materials with chlorite can remove almost all of the lignins, and the following isolation of cellulose with alkali extraction can be performed at room temperature, which has been applied to isolate cellulose from woody materials for analysis for more than a century.

In paper industry, pulping and bleaching are used to remove lignins, hemicelluloses, and other noncellulosic substances and obtain pulp fiber with high cellulose purity and brightness via chemical and mechanical processes. Chemical pulping including soda process, sulfate process, and sulfite process are the main methods to isolate cellulose fibers from lignocellulosic materials. In these procedures, NaOH, Na<sub>2</sub>S, H<sub>2</sub>SO<sub>4</sub>, Na<sub>2</sub>SO<sub>3</sub>, NaHSO<sub>3</sub> and/or SO<sub>2</sub> are

present as the major active chemicals for impregnation and delignification. The presence of high amounts of lignin in isolated cellulose fibers after delignification affects the structure and properties of the cellulose fibers. Fibers with high amounts of lignin are coarse, are stiff, and have a brownish color. Therefore, it is challenging to obtain fibers that are relatively free of bound lignin. To achieve this aim, chemical bleaching, which is used to obtain fibers with higher cellulose content from delignified and unbleached fibers, is usually considered as a continuation of delignification process to isolate cellulose from woody raw materials. Many improved and simplified techniques based on this industrial process have been proposed for isolating cellulose with high purity from straws. Moreover, many other novel procedures, including treatment with alkaline peroxide, acetic acid with nitric acid, and so on, for separating cellulose from straws have been reported in recent years.

### 5.2.1 Delignification and Alkali Extraction

Many effects have been made to isolate cellulose from various biomass sources, in which delignification and alkali extraction is considered as the most efficient method for separating cellulose from straws by releasing large amounts of lignin and hemicellulosic polysaccharides, respectively [14]. In particular, most of the lignins can be removed in a delignification step using chlorite. Delignification can significantly facilitate the extraction of the hemicelluloses during alkali treatment and therefore result in the residues of cellulosic polymers having a high purity. On the basis of the investigation of polysaccharides obtained from the delignified oat tissues, Buchala et al. [15] found about 1% of the plant tissues were lost because of the degradation and dissolution of polysaccharides during the delignification procedure. However, they pointed out that the degradation was very slight and was generally not considered. This finding indicated that delignification with chlorite does not result in noticeable degradation of hemicellulosic and cellulosic polymers. Thereafter, the hemicelluloses and cellulose can be separated from the holocellulose using alkali at room temperature [16]. Sun et al. [17] extracted cellulose from wheat straw holocellulose using 24% KOH and 2% boric acid at 20 °C for 2 h and obtained 41.8–43.0% of cellulose. Liu et al. [10] used delignification and alkali extraction to isolate cellulose from ryegrass (shown in Fig. 5.3). They found



**FIGURE 5.3** Scheme for isolation of cellulose from untreated ryegrass leaves and the dejuiced leaves [10].

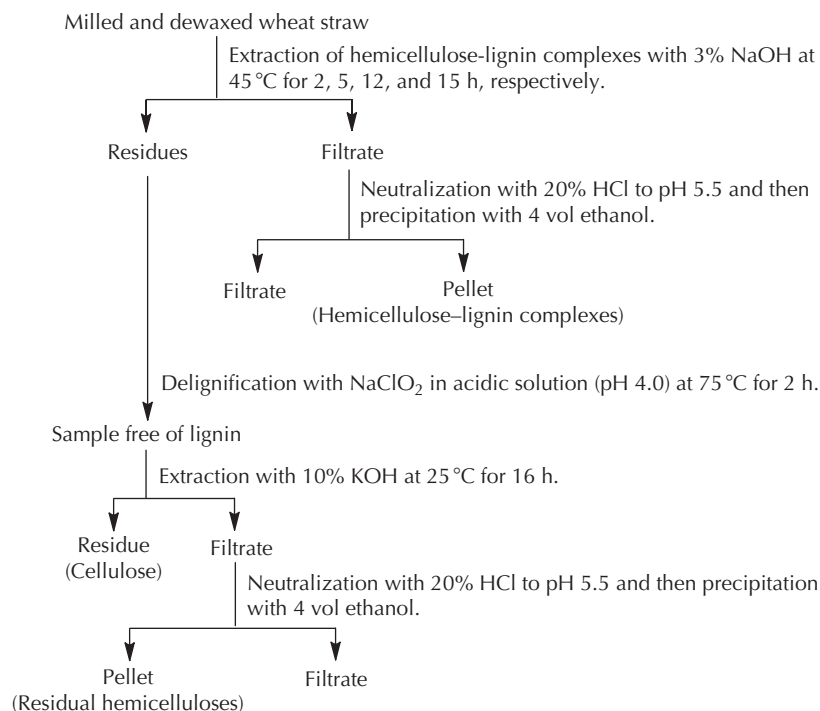
treatment at 22 °C with 18% NaOH and 18% KOH for 2 h and 10% NaOH and 10% KOH for 16 h yielded 28.2, 28.8, 22.7, and 23.4%, respectively, of cellulose residue from untreated ryegrass leaves and 35.7, 36.8, 32.8, and 34.6%, respectively, from the dejuiced ryegrass leaves. For each cellulosic fraction, the glucose content was 71.6, 69.6, 67.8, 66.7, 69.7, 68.6, 63.9, and 61.7%, respectively.

In paper industry, soda process is the main pulping method for straws because most of the lignins and hemicelluloses are dissolved in alkaline solution. Xiao et al. [18] isolated cellulose from dewaxed maize stems, rye straw, and rice straw by treatment with 1 M NaOH at 30 °C for 18 h, which resulted in dissolution of 78.0, 68.8, and 82.1% of the original lignin and 72.1, 72.6, and 84.6% of the original hemicelluloses, respectively.

Alkali treatment could extract hemicellulose-lignin complexes that are soluble in alkaline solution. Thereafter, the obtained samples undergo delignification and/or alkali extraction to isolate cellulose with relatively high purity. Sun et al. [19] isolated cellulose from dewaxed wheat straw after alkali extraction, followed by delignification and alkali extraction. Dewaxed wheat straw was first treated with 3% NaOH at 45 °C for 2–15 h with a low extractant/sample ratio. The treatments resulted in the release of 32.7–41.5% hemicellulose-lignin complexes, which contained 9.3–14.2% associated lignins. The residues of the treated straw

were sequentially delignified with NaClO<sub>2</sub> and then extracted with 10% KOH at 25 °C for 16 h. The yields of cellulose ranged 38.0–39.9%. This procedure could be used to obtain cellulosic and hemicellulosic polymers from straws and is listed in Fig. 5.4. Adinugraha et al. [20] reported a method to isolate cellulose from cavendish banana pseudo stem for further utilization. Cellulose preparations were obtained from the banana pseudo stem powder ground to pass 20 mesh after extraction with 8% NaOH at 100 °C for 3.5 h, followed by bleaching with 5% NaClO at 30 °C for 3 h. Reddy et al. [9] obtained single cells of cellulose fiber from wheat straw after dewaxing, alkali extracting with 2% NaOH solution at 95 °C for 45 min, and maceration with 10% (w/w) nitric acid and 10% (w/w) chromic acid solution. Alemдар and Sain [21] extracted cellulose nanofibers from wheat straw by chemimechanical technique of alkali and acid treatment, followed by mechanical treatments of cryocrushing, disintegration, and defibrillation. Chemical characterization of the wheat straw nanofibers confirmed that the cellulose content increased from 43 to 84%.

Interestingly, the release of noncellulosic polysaccharides from straws may be assisted by pretreatment procedures, such as ultrasonic irradiation, resulting in the cellulose with higher purity. Ultrasonic treatment is well established in the separation of plant materials, particularly for extraction of low-molecular-weight substances. The mechanical and



**FIGURE 5.4** Scheme for isolation of cellulose and hemicelluloses from wheat straw [19].

chemical effects of ultrasound are believed to accelerate the extraction of organic compounds from plant materials due to disruption of cell walls and enhanced mass transfer of the cell wall contents [22]. A sequential extraction method was developed by Liu et al. [23] with ultrasonic irradiation pretreatment to isolate cellulose from delignified SCB. The yield of original hemicelluloses increased by 4–6% with the application of ultrasonic irradiation of delignified bagasse in water for 30 min, suggesting that ultrasonic irradiation of the delignified bagasse prior to alkaline extraction offered benefits for isolation of cellulose having a high purity. The cellulosic preparations obtained were free of bound lignin and had a purity of 77.1–90.1%.

### 5.2.2 Steam Explosion

Steam explosion is one of the main methods developed recently to isolate cellulose from straws, especially for the production of bioethanol. During the steam explosion, the significant amounts of hemicelluloses are partially hydrolyzed and some lignins are depolymerized, resulting in sugars and phenolic compounds that are soluble in water. The hydrolysis of glycosidic linkages in hemicelluloses and the  $\beta$ -O-4 ether bonds in lignin are catalyzed by acetic acid formed at high temperature from acetyl groups present in hemicelluloses (autohydrolysis). On the other hand, in autohydrolysis, the depolymerized lignin fragments remain in the proximity of condensation sites in the biomass matrix [24]. The residue consists mainly of cellulose and lignin and also has some hemicelluloses. In this process, cellulose is

also depolymerized and defibrillated and undergoes a change in its crystallinity, and the resulting product is more susceptible to the hydrolytic enzymes. The lignin and residual hemicelluloses may be removed by a subsequent alkali extraction and can be recovered and used for the production of various chemicals. Therefore, the steam explosion process is generally followed by a fractionation step, such as alkaline extraction, to separate the main cellulose component. Some of the possible end products of steam-exploded lignocellulosic materials are dissolving pulp, paper pulp, ethanol, xylitol, lactic acid, and furfural or furfural derivatives [25]. It has been reported that steam explosion pretreatments improve the digestion of resulting residues and shred the fibers into many individual fragments [26]. A two-stage process proposed by Sun et al. [26] was based on the steam explosion pretreatment followed by the alkaline peroxide post-treatment for the isolation of cellulose from wheat straw. In this process, wheat straw was first steamed at 200 °C, 15 bar for 10 and 33 min, and 220 °C, 22 bar for 3, 5, and 8 min, with a solid-to-liquid ratio of 2:1 (w/w) and 220 °C, 22 bar for 5 min with a solid-to-liquid ratio of 10:1, respectively. The steamed straw was then washed with hot water to yield a solution rich in hemicelluloses-derived mono- and oligosaccharides and yielded 61.3, 60.2, 66.2, 63.1, 60.3, and 61.3% of the six abovementioned straw residues, respectively. The washed fiber was delignified and bleached with alkaline peroxide at 50 °C for 5 h under pH 11.5, which yielded 34.9, 32.6, 40.0, 36.9, 30.9, and 36.1% (% dry wheat straw) of the cellulose preparation, respectively. The optimum cellulose

yield (40.0%) was obtained when the steam explosion pretreatment was performed at 220 °C, 22 bar for 3 min with a solid-to-liquid ratio of 2:1, in which the cellulose fraction obtained had a viscosity-average degree of polymerization (DP) of 587 and contained 14.6% hemicelluloses and 1.2% Klason lignin. The steam explosion pretreatment led to a significant loss in hemicelluloses, and the following alkaline peroxide post-treatment resulted in substantial dissolution of lignin and an increase in cellulose crystallinity.

### 5.2.3 Alkaline Peroxide Extraction

It is well known that the hydrogen peroxide reacts with lignin under alkaline conditions and has been widely used for many years to bleach high-lignin wood pulps. The bleach effect of hydrogen peroxide has been attributed to its ability to react with various colored carbonyl-containing structures in lignin. This reaction has been explained through the reactions of the hydroperoxide anion ( $\text{HOO}^-$ ), formed in an alkaline medium. On the other hand, depending on the pH, hydrogen peroxide acts as either a nucleophilic or an electrophilic species. It is stable under acidic conditions. Above pH 6.0, it is unstable and readily decomposes, particularly in the presence of certain transition metals such as iron, copper, and manganese. Decomposition of the hydrogen peroxide forms molecular oxygen and more active radicals such as the hydroxyl radicals ( $\text{HO}^\bullet$ ) and superoxide anion radicals ( $\text{O}_2^{\bullet-}$ ), which in turn react with lignin in a variety of ways, thus resulting in delignification by both degradation and dissolution [17, 18]. Alkaline peroxide extraction exhibits good performance in isolation of cellulose from straws, but less effective from wood.

Recently, one of the most important approaches to cellulose isolation from straws is based on the hydrogen peroxide treatment in alkaline solution. Sun et al. [27] found that the extraction of the dewaxed wheat straw using 2%  $\text{H}_2\text{O}_2$  2% NaOH aqueous solution for 5 h at 45 °C and 50 °C resulted in dissolution of 85 and 86% of the original lignin and 75 and 76% of the original hemicelluloses, respectively, and left 53.8 and 53.3% cellulose. Fang et al. [28] compared the extraction of water-treated rye straw with alkali and alkaline peroxide to isolate cellulose and hemicelluloses. The results showed that the treatment of the straw with the dilute alkaline solution at pH 11.5 for 12 h at 50 °C in the absence of  $\text{H}_2\text{O}_2$  yielded only 7.3% of the original hemicelluloses and 7.4% of the original lignin, whereas extraction with 2%  $\text{H}_2\text{O}_2$  at pH 11.5 for 12 h at 20–70 °C released 44.2–71.9% of the original hemicelluloses and 52.7–87.8% of the original lignin.

### 5.2.4 Organic Solvent Extraction

The processes currently used for commercial straw pulping using inorganic reagents achieve high cellulose extraction efficiency only at the expense of the hemicellulosic fraction,

which undergoes hydrolysis and degradation. These processes not only underexploit the lignin but also cause serious environmental problems. For these reasons, intensive research is being carried out on the development of environmentally friendly approaches, which generally involve the use of organic solvents for efficient separation of the cell wall components [29].

The organosolv pulping processes involve the treatment of lignocellulosic substances with organic solvent/water media in the presence or absence of a catalyst because they have lower environmental impact and lower energy consumption. The fractions of cellulose, hemicelluloses, and lignin obtained have different characteristics depending on the specific process conditions [30]. Organic acid pulping such as acetic acid pulping has been proved to be an effective method to delignify and fractionate straws [31–34]. Lam et al. [35] studied rice straw pulping using formic acid. Approximately 85% of delignification with a cellulose pulp yield of 44.4% was obtained under relatively mild cooking conditions (temperature, 100 °C; cooking time, 60 min; and formic acid concentration, 90%). They thought the advantage of this technique compared with cooking in basic environments was that most of the silicon derivatives remain in the pulp. Recently, one of the developments in acetic acid pulping is the FORMACELL process, based on the addition of 5–10% formic acid to aqueous acetic acid, resulting in improved selectivity of delignification [36]. Besides the role of delignification, organic acids actively take part in the hydrolysis of hemicelluloses. Correspondingly, organic acid based pulping processes include the option for the manufacture of dissolving pulps that are used as a feedstock for cellulose derivatives and cellulosic fibers [37]. A method based on an organic acid treatment to isolate cellulose from wheat straw proposed by Sun et al. [29] includes a two-stage acidic organosolv treatment followed by a cyanamide-activated hydrogen peroxide bleaching. The effects of concentration of acetic and formic acids were investigated on the yield of cellulose and degradation of lignin and noncellulose polysaccharides. Organic acids were more effective than alcohols on the degradation of lignin and hemicelluloses. Formic acid/acetic acid/water (30/60/10, v/v/v) system was found to be the most effective in delignification and removal of noncellulose polysaccharides from the straw and did not have any undesirable effects on cellulose properties such as its intrinsic viscosity. The treatment using 0.1% HCl as a catalyst at 85 °C for 4 h removed 94.1% of the original lignin and 76.5% of the original hemicelluloses.

The treatment with 80% acetic acid using 70% nitric acid as a catalyst (10:1, v/v) described by Crampton et al. [38] and Brendel et al. [11] is another recommended method based on organic acid to isolate cellulose from straws. Sun et al. [39] found that wheat straw lignins and hemicelluloses were degraded in the medium containing 80% acetic acid and 0.92–13.5% nitric acid. The treatment with 80% acetic



acid and 0.92% nitric acid as a catalyst at 120 °C for 20 min resulted in more than 81% original hemicelluloses and 92% original lignin degradation. As the nitric acid concentration increased to 8.5%, more than 96% original hemicelluloses and approximately 98% original lignins were degraded and yielded the cellulose approaching 96% purity. Xu et al. [40, 41] used this procedure to isolate cellulose from wood and cereal straws. The results showed that the treatment using 80% acetic acid with 2.0–8.0% (w/w) nitric acid as a catalyst led to the significant degradation of lignins and hemicelluloses, the slight acetylation of cellulose, and an increase in the degree of crystallinity of cellulose. Glucose was comprised with more than 90% of the total neutral sugars of the isolated cellulose preparations, revealing the relatively high purity of cellulose samples.

Sun et al. [42] comparatively studied three different procedures including alkaline peroxide with or without ultrasonic treatment, delignification with acidified sodium chlorite followed by alkaline extraction with KOH or NaOH, and treatment with 80% acetic acid–70% nitric acid mixture (10:1, v/v) for isolation of cellulose from SCB. The results showed that alkali and alkaline peroxide with or without ultrasonic treatment yielded 44.7 and 45.9% cellulose preparations, which contained 6.0 and 7.2% associated hemicelluloses and 3.4 and 3.9% bound lignins, respectively. Delignification with acidic sodium chlorite followed by extraction with alkali (10% KOH and 10% NaOH) resulted in the cellulose yields of 44.7 and 44.2%, which contained 5.7 and 3.7% residual hemicelluloses and 1.6 and 1.5% remaining lignins, respectively. One-step treatment of SCB with an 80% acetic acid–70% nitric acid mixture under the given conditions yielded 43.0–43.6% of more pure cellulose fractions, which contained minor amounts of bound hemicelluloses (3.2–4.3%) and were relatively free of associated lignin (0.2–0.6%). This finding indicated that organic acid treatment with nitric acid as a catalyst is more effective than alkaline peroxide and delignification followed by alkaline extraction for isolation of cellulose with high purity from straws.

However, higher requirement for equipments is needed in organic acid treatments because of the corrosion. The procedure combining organic acids with alkali and alkaline peroxide treatment is preferable. Sun et al. [43] proposed a sequential totally chlorine-free procedure for isolation of cellulose from wheat straw based on the alkaline extraction in organic alcohol, peroxide treatment, and purification with 80% acetic acid–70% nitric acid. It was shown that pretreatment with 0.5 M NaOH in 60% methanol at 60 °C for 2.5 h, under ultrasonic irradiation for 0–35 min, and subsequent post-treatment with 2% H<sub>2</sub>O<sub>2</sub>–0.2% TAED at pH 11.8 for 12 h at 48 °C solubilized 85.3–86.1% of original hemicelluloses and 91.7–93.2% of the original lignins, respectively. The crude cellulose with the yield between 46.2 and 49.2% on a dry weight of wheat straw contained 11.2–12.2% residual hemicelluloses and 2.5

2.9% remaining lignins. Further treatment of the corresponding crude cellulosic preparations with 80% acetic acid–70% nitric acid yielded 36.8–37.7% of the cellulose with high purity. This procedure is shown in Fig. 5.5.

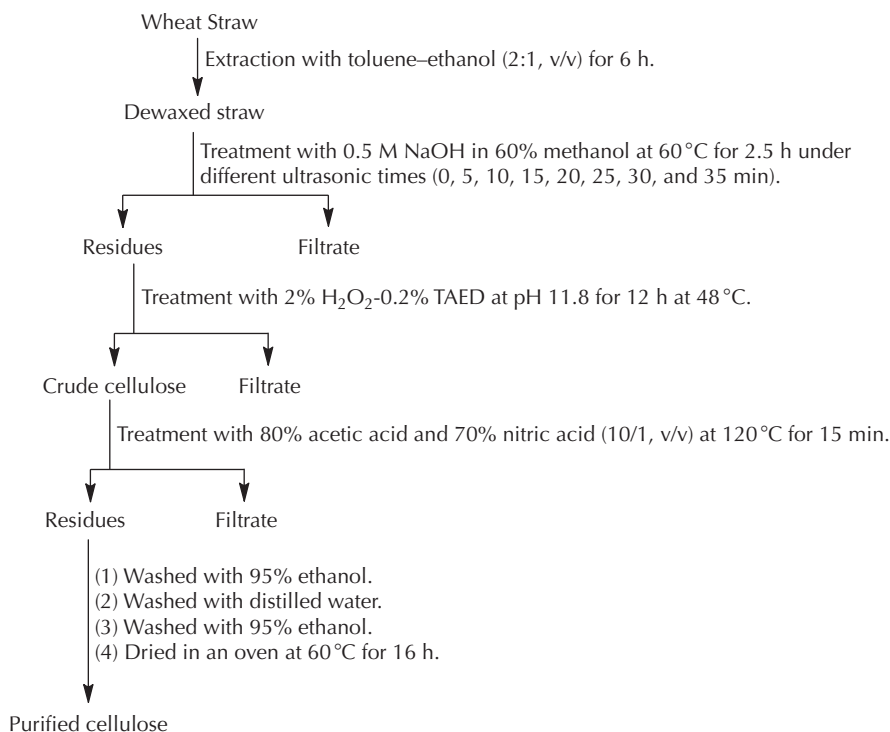
Similar procedure was used to isolate cellulose from many kinds of lignocellulosic materials including barley straw, oil palm frond fiber, poplar wood, maize stems, wheat straw, rice straw, and rye straw, as shown in Fig. 5.6 [44–46]. Highly purified cellulose preparations were obtained by pretreatment with 2.0% H<sub>2</sub>O<sub>2</sub> at 45 °C and pH 11.6 for 16 h and sequential purification with 80% acetic acid–70% nitric acid (10/1, v/v) at 120 °C for 15 min. The purified cellulose obtained was relatively free of bound hemicelluloses (2.3–3.2%) and lignins (0.4–0.6%) and had a yield of 35.5% from barley straw, 39.6% from oil palm frond fiber, 40.8% from poplar wood, 36.0% from maize stems, 34.1% from wheat straw, 23.4% from rice straw, and 35.8% from rye straw, respectively, as shown in Table 5.1 [46].

Treatment with 80% acetic acid–70% nitric acid resulted in not only the removal of hemicelluloses and lignins but also a noticeable degradation of the cellulose. The following is the sequential treatments of dewaxed straw: 0.5 M aqueous KOH at 35 °C for 2.5 h under ultrasonic irradiation time of 0 to 35 min, 2% H<sub>2</sub>O<sub>2</sub>–0.2% TAED at pH 11.8 for 12 h at 48 °C, and 80% acetic acid–70% nitric acid (10/1, v/v) at 120 °C for 15 min. The yield of crude cellulose preparations obtained by first two-stage treatments ranged between 45.3 and 46.9% of the dry-weight straw, which contained 7.3–7.9% residual hemicelluloses and 3.3–3.7% residual lignins, and their molecular weights that ranged from 269–960 to 258–280 g mol<sup>−1</sup> were determined by their viscosity, whereas the purified cellulose samples separated by a further treatment of the corresponding crude cellulose with 80% acetic acid–70% nitric acid are relatively free of bound lignin (0.1 to 0.2%) and contained minor amounts of associated hemicelluloses (~3%), but it yielded much lower molecular weights ranging between 42–300 and 44–650 g mol<sup>−1</sup> [45], indicating the noticeable degradation of cellulose.

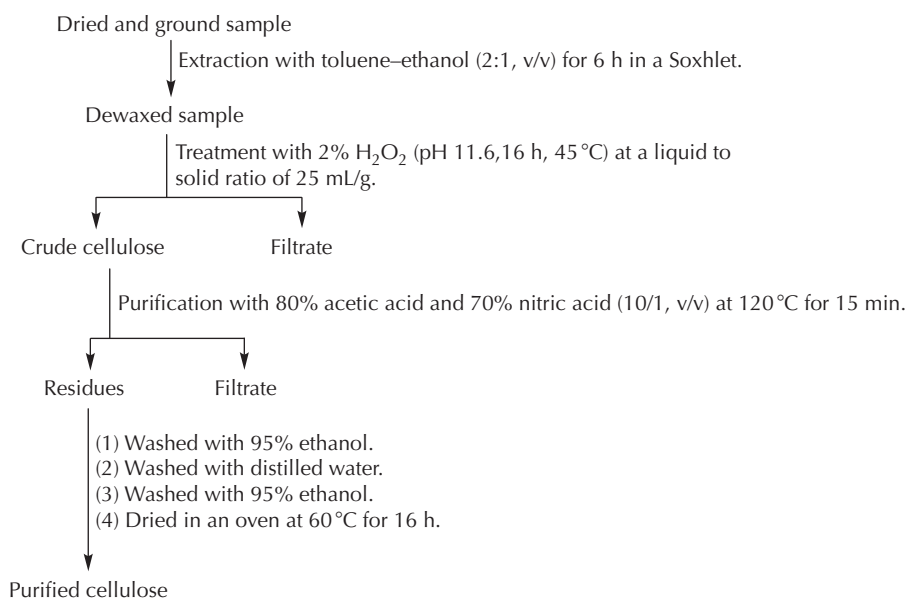
## 5.2.5 Other Isolation Methods

There are many other methods, including acid hydrolysis and biological treatment, for the isolation of cellulose from straws. Zhao et al. [47] proposed an integrated process for the isolation of nearly pure cellulose from rice straw based on treatment with dilute acid to decompose hemicelluloses followed by delignification with sulfomethylation reagents. Bhattacharya et al. [48] isolated cellulose microfibers from SCB using a conventional pulping process to eliminate lignin and hemicelluloses, mechanical homogenization, and acid hydrolysis. Hydrolysis of the cellulose fibers with 60% (w/v) sulfuric acid for 2.5 h at 60 °C was found to be optimum and resulted in the removal of most of the amorphous domains without any significant damage to the crystal structure. Kikuchi et al. [49] used biological





**FIGURE 5.5** Scheme for isolation of crude and purified cellulose from wheat straw [43].



**FIGURE 5.6** The scheme for isolation of crude and purified cellulose from dewaxed barley straw, oil palm frond fiber, poplar wood, maize stems, wheat straw, rice straw, and rye straw [46].

treatment with molecular-genetically bred *Coprinus cinereus* monokaryotic strains to isolate cellulose efficiently from rice straw. The results showed that the recoveries of the cellulose could increase up to 29%. Ates et al. [50] comparatively studied the different pulping processes including kraft-anthraquinone (AQ), bio-kraft, soda-AQ, ALCELL, and FORMACELL for wheat straw. Fungal pretreatment with

*Ceriporiopsis subvermisporea* (*C. subvermisporea*), a kind of white rot fungi, was applied to wheat straw before kraft-AQ pulping, the so-called bio-kraft process. The results indicated that kraft-AQ pulps from wheat straw exhibited better characteristics than the other pulp samples, such as lower lignin content, higher carbohydrate content, higher paper strength properties, and better bleachability. The highest

**TABLE 5.1** Yield of Crude and Purified Cellulosic Preparations (% Dry Matter) Isolated by Treatment of Various Cellulosic Materials [46]

	Barley straw	Oil palm frond fiber	Poplar wood	Maize stems	Wheat straw	Rice straw	Rye straw
Cell corner	58.4	58.2	78.3	57.5	47.3	38.4	52.4
Content of hemicelluloses in cell corner	12.3	7.5	17.5	10.6	10.6	6.8	14.5
Content of lignin in cell corner	7.8	806	21.8	3.4	4.9	3.6	3.5
Purified cellulose	35.3	39.6	40.8	36.0	34.1	23.4	35.8

kappa number, viscosity, and fiber coarseness were found for organosolv pulp samples; however, these pulps had the lowest carbohydrate contents and strength values and poor bleaching properties. It was concluded that the fungal pretreatment of wheat straw with *C. subvermispora* had a positive effect on the bleachability and yielded stronger pulp.

## 5.3 STRUCTURE AND CRYSTALLINE LATTICE OF CELLULOSE I

### 5.3.1 Supermolecular Structure

The chemical structure of cellulose has been clarified in detail. It is a linear homopolymer composed of D-glucopyranose units linked by  $\beta$ -1,4-glycosidic bonds. This is explained by the presence of three hydroxyl groups with different polarities, secondary OH at the C-2, secondary OH at the C-3, and primary OH at the C-6 position and accordingly, by the formation of various strong intermolecular and intramolecular hydrogen bonds [51]. The schematic hydrogen bonding in cellulose structure is shown in Fig. 5.7.

Because of the strong tendency for intra- or intermolecular hydrogen bonding, bundles of cellulose molecules aggregate to microfibrils, which form either highly ordered (crystalline) or less ordered (amorphous) regions [53]. Microfibrils are further aggregated to fibrils and finally to cellulose fibers. The tight fiber structure created by hydrogen bonds results in the typical material properties of cellulose, such as high tensile strength and insolubility in most solvents. However, at present, supermolecular state and polymer properties of cellulose are not yet fully understood. X-ray and other diffraction methods have played a decisive role in the analysis of the crystalline structure of cellulose. Even though there are still contradictory opinions, the view that the chains of native cellulose are parallel has been generally accepted. The crystalline and amorphous regions are seen in Fig. 5.8.

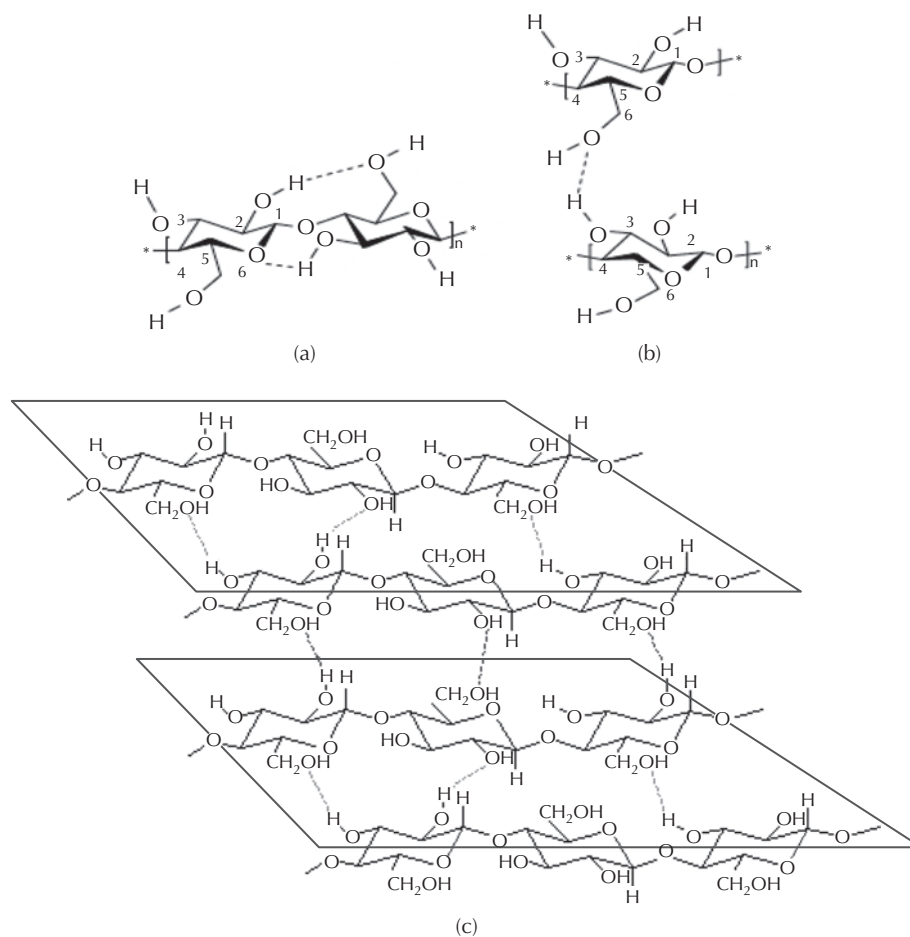
There are significant differences between the properties of straw cellulose, wood cellulose, and cotton cellulose. The cellulose crystallites are longer in straw pulps than in wood

pulps, but they are not as long as in cotton cellulose. In addition, the degree of crystallinity of straw pulps appears to be less than that of wood cellulose. Low crystallinity can be useful when a cellulose derivative is to be manufactured.

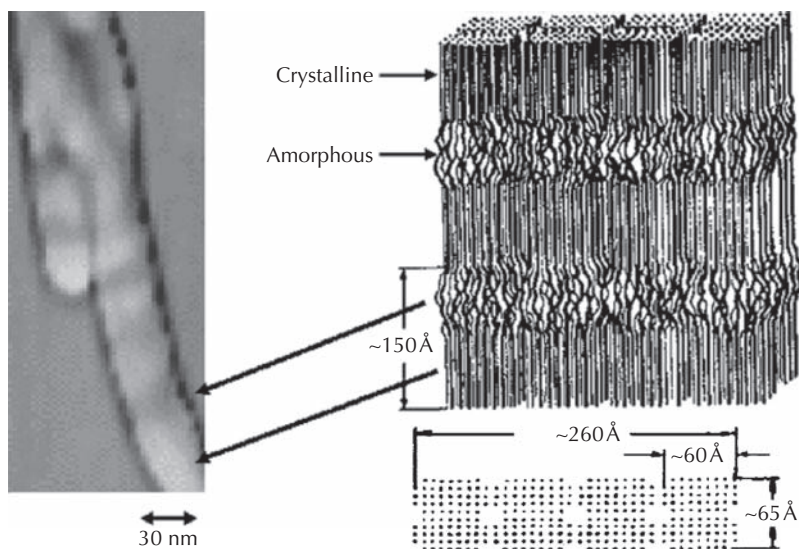
### 5.3.2 Cellulose Lattice I

Despite its simple molecular structure, cellulose shows a large complexity and variability in its supermolecular arrangement in cellulose fibrils, which are surrounded by a matrix of lignins and hemicelluloses. The crystalline cellulose is known to crystallize in several different polymorphs. On the basis of X-ray diffraction (XRD) patterns and  $^{13}\text{C}$ -nuclear magnetic resonance ( $^{13}\text{C}$ -NMR) spectra, four major polymorphs of cellulose have been reported, namely cellulose I, II, III, and IV. The most abundant native crystalline form is cellulose I, whereas cellulose II, III, and IV are synthetic crystalline cellulose allomorphs, which vastly differ from native cellulose in their atomic conformational structure [54, 55].

Cellulose I is the native and predominant crystalline structure of cellulose in alga, bacteria, some animal, and most high plants [56]. The crystallites in native cellulose are very small, typically 20–50 Å in diameter, which cause considerable peak broadening and serious peak overlap. The crystallite diameter can be determined with XRD based on the width of peaks representing directions perpendicular to the fiber axis ((110), (1 $\bar{1}$ 0), and (200) planes), whereas the crystallite length can be determined based on the width of peaks representing directions parallel with the fiber axis ((004) plane), as shown in Fig. 5.9. Liu et al. [23] found that all of the cellulosic preparations have the typical cellulose I structure, but the crystallinity of the SCB cellulose was lower than that of flax, cotton, and kenaf. El-Sakhawy et al. [57] prepared microcrystalline cellulose (MCC) from bagasse, rice straw, and cotton stalks bleached pulps. They found that all samples have a typical crystal lattice for cellulose I, and all MCC samples had similar crystallinity index values with slightly lower values for bagasse MCC. However, rice straw had the smallest crystallite size



**FIGURE 5.7** Cellulose structure showing (a) the intramolecular hydrogen bonding between C2 OH and C6 OH and C3 OH with endocyclic oxygen, (b) the intermolecular bonding between C3 OH and C6 OH, and (3) the intermolecular bonding between C2 OH and C6 OH and C3 OH [52]. (see color plate)



**FIGURE 5.8** Atomic force microscopy phase images support the presence of crystalline and amorphous regions in the direction of the fiber axis [48]. (see color plate)

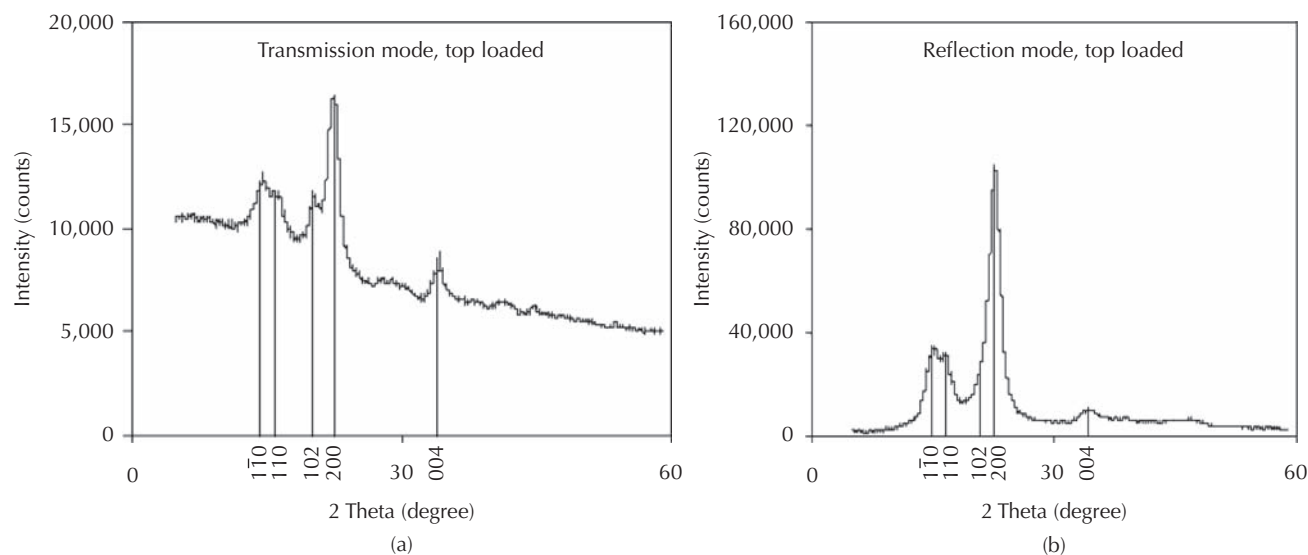


FIGURE 5.9 Diffractograms of cellulose I measured in transmission mode (a) and reflection mode (b) [59].

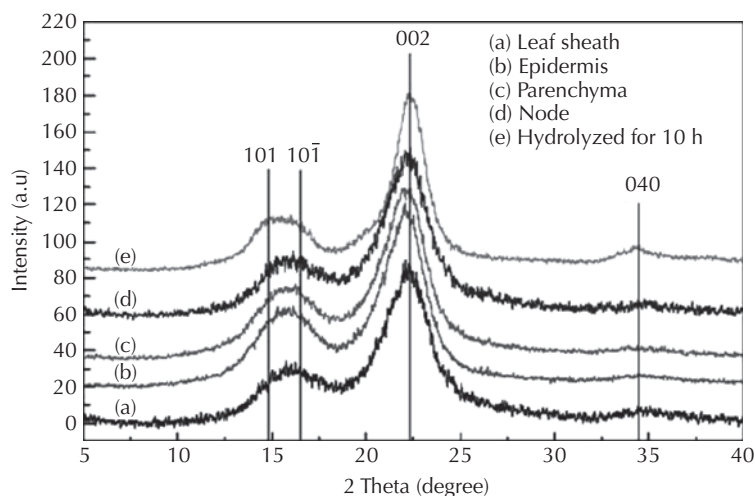


FIGURE 5.10 Wide angle X-ray diffraction curves of cellulose from wheat straw [60].

followed by bagasse and cotton stalks. In native cellulose (cellulose I), the cellulose polymer chains are stacked together during biosynthesis in polymer bundles known as fibrils or microfibrils [58]. Figure 5.10 shows the wide-angle X-ray diffraction (WAXD) curves of cellulose samples from different parts, that is leaf sheath, epidermis, parenchyma, and node of the wheat straw after the extraction of lignins and hemicelluloses. The WAXD curves of all the cellulose samples from the wheat straw show typical XRD patterns of semicrystalline cellulose I allomorph with low crystallinity. The diffraction peaks of the (101) and (10 $\bar{1}$ ) planes merged together, whereas the diffraction peak (040) is very weak. Moreover, the crystallinities of cellulose from different parts of wheat straw are little different and fairly low, about 40%, as listed in Table 5.2. When the cellulose from wheat straw was hydrolyzed by 60% H<sub>2</sub>SO<sub>4</sub> aqueous solution at 70 °C for 10 h, during which the amorphous part

of cellulose is hydrolyzed, the crystallinity of cellulose obtained increased to 77.2% and the crystals of cellulose I became more perfect. The diffraction peaks of the (101) and (10 $\bar{1}$ ) planes can be resolved in the WAXD curve, and the diffraction peak of the (040) plane appeared (Fig. 5.10).

Cellulose I exists as a mixture of two different crystalline forms, cellulose I $_{\alpha}$  and cellulose I $_{\beta}$ . Both are frequently found to coexist in cell wall structures together with amorphous cellulose [61, 62]. Focher et al. [63] investigated cellulose from flax, hemp, kenaf, and sorghum by solid state NMR, vibrational spectroscopy, and XRD, and the results revealed the presence of I $_{\alpha}$  and I $_{\beta}$  forms. Cellulose I $_{\alpha}$  has one-chain triclinic structure and cellulose I $_{\beta}$  has two-chain monoclinic structure [64], and they differ in hydrogen bonding. A sheet-like structure composed of intermolecular hydrogen bonds running parallel to the pyranose rings of the parallel cellulose chains is common to

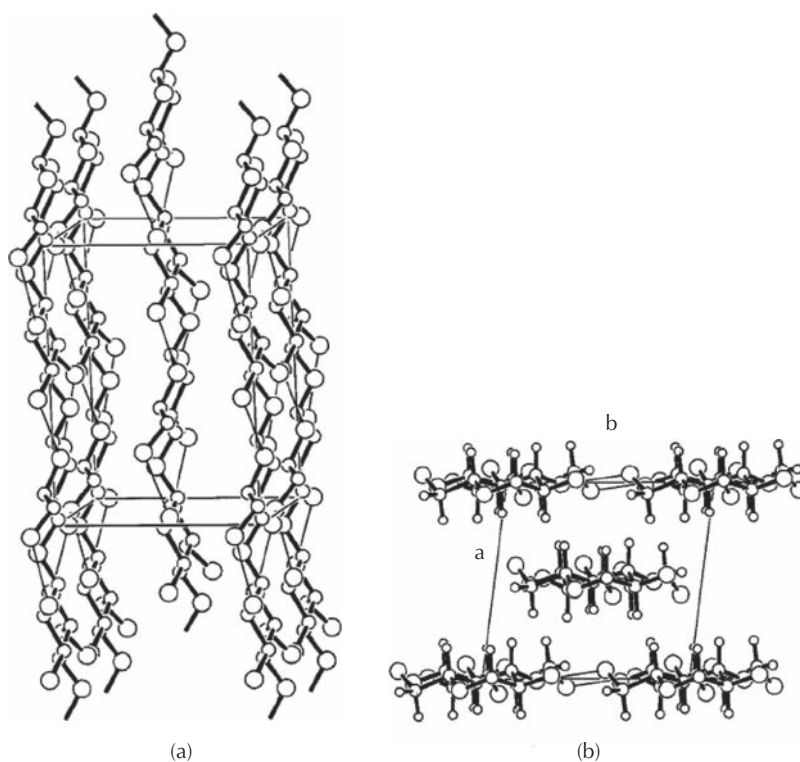
**TABLE 5.2** Crystallinity Index of Cellulose from Different Parts of Wheat Straw [60]

Sample	Crystallinity (%)
Leaf sheath	47.4
Epidermis	44.4
Parenchyma	44.3
Node	43.2
Hydrolyzed for 10 h	77.2

both forms, but the sheets are stacked differently in cellulose  $I_\alpha$  and  $I_\beta$ . In cellulose  $I_\alpha$ , the sheet stacks are linked via van der Waals' interactions and have a progressive shear running parallel to the chain axis. In cellulose  $I_\beta$ , the sheet stacks have an alternating shear [65, 66]. Cellulose  $I_\alpha$  is metastable, and cellulose  $I_\beta$  is more stable [67]. Cellulose  $I_\alpha$ , the rare form, has been reported to naturally exist only in some green algae with relatively large amount along with some cellulose  $I_\beta$ , whereas cellulose  $I_\beta$  is predominant in higher plants such as straws and wood. It is also known that cellulose  $I_\alpha$  can be irreversibly converted to the more stable form  $I_\beta$  by heating [67–70]. These allomorphs are believed to coexist in the fibril with a different ratio, depending on the origin. Further, in the fibril,

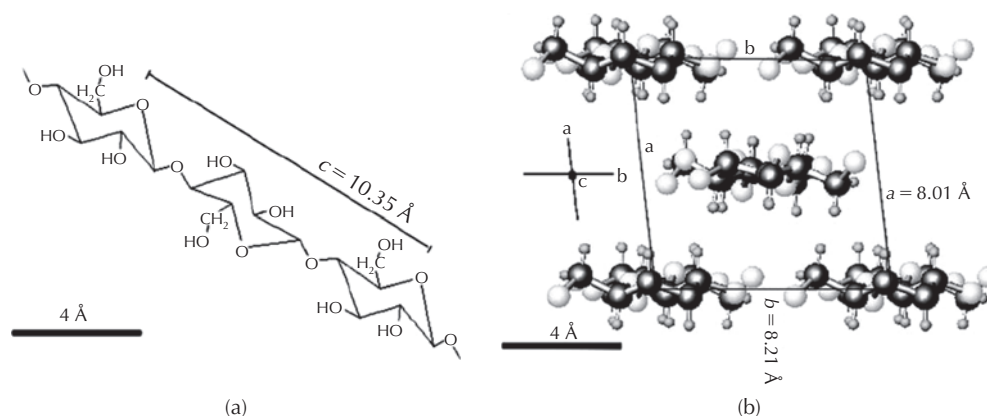
the cellulose chains exist in regions of various degree of order. Noncrystalline cellulose is also present in the fibril; paracrystalline cellulose and cellulose are present at inaccessible and accessible fibril surfaces, respectively. It may be present as distortions in the cellulose lattice, and cellulose chains located on the fibril surface are not locked in a three-dimensional structure [71].

The crystal structure of cellulose in higher plants including straws is that of cellulose  $I_\beta$ , the monoclinic two-chain unit cell, in which  $c$ -axis of the unit cell is parallel to the fiber axis, and the space group is approximated to  $P2_1$  [59], as shown in Figs. 5.11 and 5.12. Cellulose in straws is rather disordered and has a low crystalline structure of cellulose  $I_\beta$  [72]. Li et al. [73] studied crystalline cellulose in the cell walls of straw by atomic force microscopy (AFM). Two allomorphs of crystalline cellulose, triclinic  $I_\alpha$  and monoclinic  $I_\beta$  phases, were identified from their different morphological features appearing in the high-resolution AFM images. The results showed that in most crystalline regions, the  $I_\alpha$  and  $I_\beta$  phases are intimately associated, with the  $I_\beta$  phase more abundant than the  $I_\alpha$  phase. In some small domains, only one phase with long-range order was observed. It was demonstrated that in these one-phase domains,  $I_\alpha$  phase crystals always have their (010) plane lying parallel to the cell wall surface and  $I_\beta$  phase crystals with (110) plane lying parallel. Cellulose crystallizes through hydrogen bonding between the chains and has cellobiose as repeated unit.



**FIGURE 5.11** Views of the refined cellulose  $I_\beta$  crystal structure (a) obliquely to and (b) along the  $c$  axis. Thin lines show hydrogen bonds. The hydrogen atoms are excluded from part (a) for clarity [74].





**FIGURE 5.12** (a) The repeated cellobiose unit in cellulose as compared to the  $c$  axis length. (b) The cellulose  $I_\beta$  structure viewed along the unique  $c$  axis of the  $P2_1$  unit cell with the  $a$  and  $b$  axis in the paper plane (five ends of molecule chain are thereby shown) [59].

### 5.3.3 Conversion of Cellulose I to Cellulose II

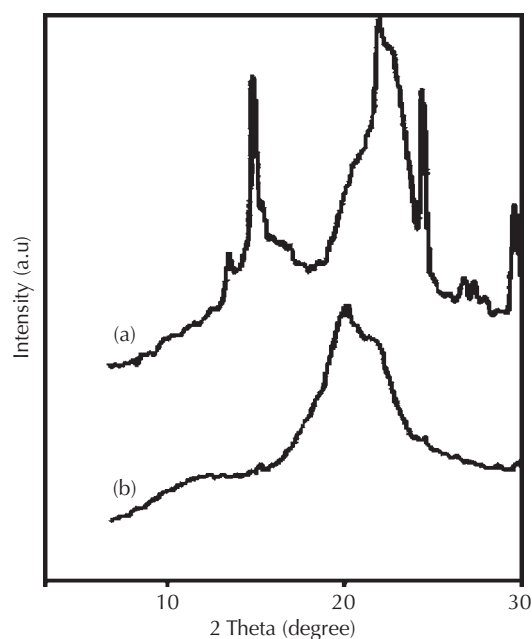
It is well established that when subjected to strong alkali solutions, crystalline native cellulose, cellulose I, becomes swollen and upon washing shrinks back to yield a new allomorph, cellulose II [56, 75]. For example, mercerization of the cellulose fiber in aqueous solution of NaOH breaks hydrogen bonds and weak van der Waal's forces between the cellulose chain molecules and results in reorganization to cellulose II when the swelling agent is removed [76]. At present, it seems generally accepted that the cellulose I structure is made of parallel chains [74], whereas the crystal structure of cellulose II is described as antiparallel [51, 77, 78]. The observations of the mercerization of isolated cellulose microfibrils from sugar beet pulp were consistent with this concept of cellulose microfibrils made of parallel chains in cellulose I and antiparallel chains in cellulose II [75]. Figure 5.13 shows the typical XRD pattern of cellulose I and cellulose II. In cellulose I, strong crystalline peaks at  $14.8^\circ$ ,  $16.8^\circ$ , and  $22.6^\circ$  correspond to the (110), ( $1\bar{1}0$ ), and (200) planes of crystals [79], whereas in cellulose II, a broad crystalline peak at around  $19.8^\circ$  is due to (110) plane of cellulose crystals [79]. Zuluaga et al. [80, 81] found that the cellulose microfibrils isolated with peroxide alkaline, peroxide alkaline hydrochloric acid, or 5 wt% potassium hydroxide from banana rachis was cellulose  $IV_1$  or cellulose  $I_\beta$ , whereas that treated with a more concentrated KOH solution (18 wt%) was converted to cellulose II. The XRD spectrum is shown in Fig. 5.14. This finding indicated that the parallel-to-antiparallel reorganization of cellulose chains could be initiated in the amorphous regions of alkali-swollen microfibrils. The molecules from adjacent cellulose I microfibrils with opposite chain polarity could then rearrange and crystallize into antiparallel cellulose II upon washing in water.

Cellulose I can also be converted to cellulose II by regeneration of dissolved cellulose. The cellulose regenerated from DMAc/LiCl solutions after complete dissolution has been reported to form the cellulose II crystal form [82–84]. Hattori et al. [85] found that cellulose II and amorphous cellulose were

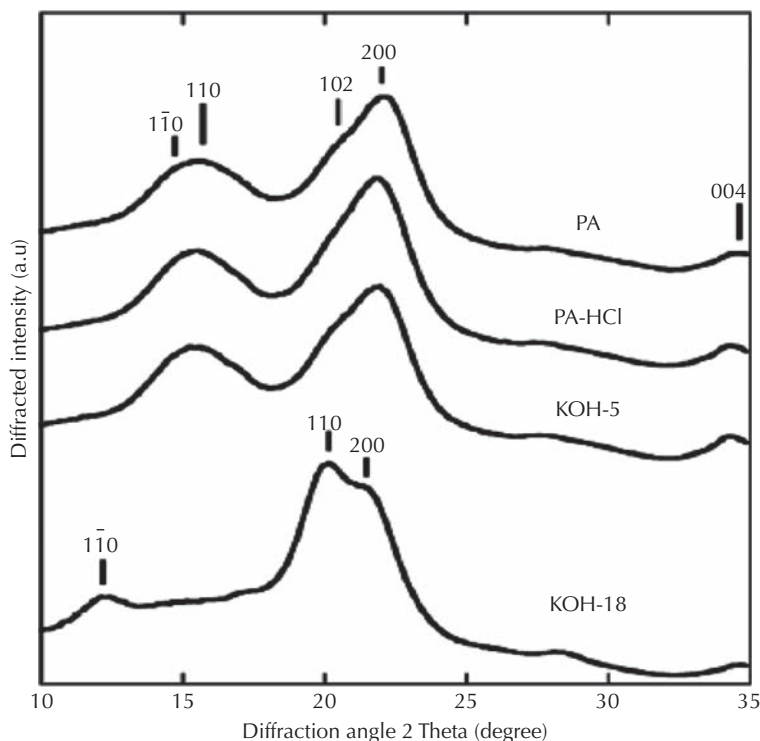
recovered from the cellulose/ethylenediamine/thiocyanate salt solutions when water and alcohol were used as a coagulant, respectively. In addition, small amounts of cellulose II has been observed to form during kraft pulping [86].

Microwave irradiation could reduce the concentration of NaOH in the solution and the time of treatment, which are needed for the complete transformation of cellulose lattice type I into cellulose lattice type II without any heating, and produce no considerable effects on the mechanism of mercerization.

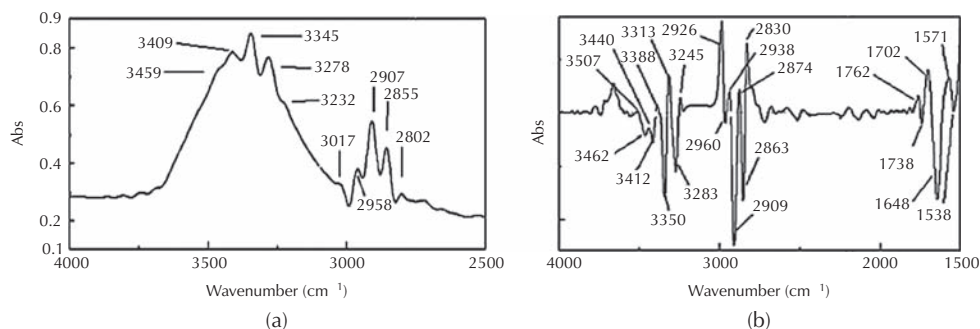
In addition, it has been proved that the deconvolution and the second derivatives of the Fourier transform infrared (FTIR) spectra of fibers can be used as a useful tool for distinguishing cellulose lattice type II from cellulose lattice type I (Fig. 5.15) [87]. Moharram and Mahmoud [76, 87]



**FIGURE 5.13** X ray diffraction pattern of cellulose I (a) and cellulose II (b) [20].



**FIGURE 5.14** XRD spectra recorded from films of cellulose microfibrils prepared by different treatments: peroxide alkaline (PA), peroxide alkaline hydrochloric acid (PA HCl), potassium hydroxide 5 wt% (KOH 5), and potassium hydroxide 18 wt% (KOH 18). The indexation is that defined in Sugiyama et al. [64].



**FIGURE 5.15** (a) The deconvolution of FTIR spectrum of cellulose I. (b) Second derivatives of FTIR spectrum of cellulose I [87].

studied the transformation of cellulose I into cellulose II during mercerization with microwave heating using XRD and FTIR spectroscopy techniques.

## 5.4 PHYSICO-CHEMICAL PROPERTIES

### 5.4.1 Structural Properties

Cellulose synthesized by nature can be considered a quite perfect molecule: anhydroglucose units (AGU) are connected by  $\beta$ -1,4-glycosidic linkages resulting in a homopolymer with three hydroxyl groups per AGU and a terminal aldehyde masked as hemiacetal at the reducing end [88], as shown in Fig 5.2.

For isolation and utilization of cellulose from straws in a pure and undergraded form, its polymer properties need to be studied; however, no reasonably simple methods for elucidation of polymer properties are available. There are many technologies to characterize the physicochemical properties of cellulose, including molecular weight determination, FTIR, NMR, and XRD.

The molecular weight of cellulose can be defined by its average DP. The average molecular mass results from the DP and the molecular mass of AGU. The determination of DP is usually performed viscosimetrically after dissolving the samples in solvents, of which the most common are cupriethylenediamine (CED) and cadmiummethylenediamine (Cadoxen). The polydispersity of native straw cellulose is

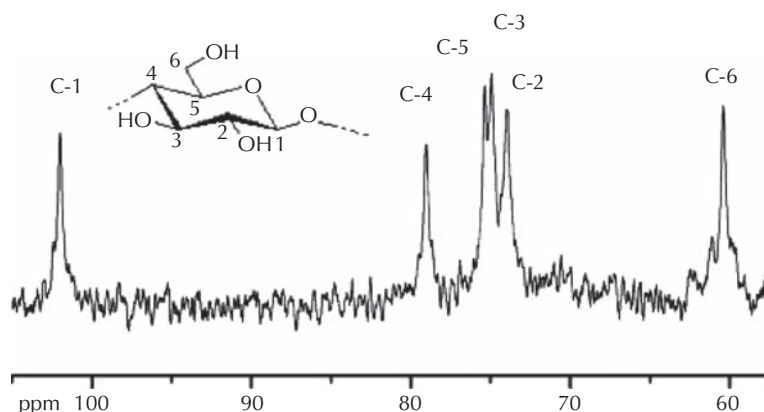


FIGURE 5.16  $^{13}\text{C}$  NMR solution spectrum of cellulose [90].

**TABLE 5.3** Assignment of  $^{13}\text{C}$ -NMR Signals of Unsubstituted Cellulose

C-atom	Assignment (ppm)
C 1	104.5
C 2	74.7
C 3	76.1
C 4	79.8
C 5	76.3
C 6	61.5

probably quite low, which means that  $\text{DP}_n$  and  $\text{DP}_w$  do not deviate much from each other. Measurements of its polymer properties indicate that cellulose in solution belongs to the groups of randomly coiling polymers. The high viscosity and the other polymer properties show that cellulose is a solvent-swollen polymer in contrast to lignin, which in solution occupies a compact structure. The DP of cellulose samples isolated from straws differs widely in the range of 500–2500, depending on the origin, isolation method, and determination method. Six cellulosic preparations isolated with alkali extraction from SCB after dewaxing with chloroform ethanol and delignifying with chlorite, followed by ultrasonic irradiation, had a purity of 77.1–90.1% with DP ranging from 1858.1–2238.2 [23]. The DP of cellulose preparations isolated from steam-exploded wheat straw ranged from 800 to 1660; after post-treatment with alkaline peroxide at 50 °C for 5 h, it decreased to 532 to 608 [26].

The FTIR spectrum of cellulose is always similar except for the intensity of absorption bands. In the spectrum, the absorption at 2900  $\text{cm}^{-1}$  relates to the CH and  $\text{CH}_2$  stretching and the one at 1372  $\text{cm}^{-1}$  to the O-H bending. The peak at 893  $\text{cm}^{-1}$  represents the glycosidic  $\text{C}_1\text{-H}$  deformation with ring vibration contribution, which is characteristic of

$\beta$ -glycosidic linkages between glucose in cellulose. A peak at 1426  $\text{cm}^{-1}$  relates to the  $\text{CH}_2$  symmetric bending. The total crystallinity (TCI) and lateral order indices (LOI), proposed by Nelson and O'Connor, can be obtained from the 1429/893 and 1372/2900  $\text{cm}^{-1}$  absorbance ratios, respectively. TCI and LOI represent the relative crystallinity degree, and the changes of absorbance ratios can reflect the cellulose crystallinity changes. Based on this method, Liu and Chen [89] investigated the change of cellulose treated with IL  $[\text{C}_4\text{mim}]\text{Cl}$ . The results showed that the crystallinity of wheat straw and steam-exploded wheat straw treated with  $[\text{C}_4\text{mim}]\text{Cl}$  decreased with increasing treatment time.

Furthermore, the absorption bands at 750 and 710  $\text{cm}^{-1}$  in FTIR spectrum of cellulose are assigned to the  $\text{I}_\alpha$  and  $\text{I}_\beta$  phases, respectively [80]. In the FTIR spectrum of cellulose extracted from the wheat straw, the absorption band at 750  $\text{cm}^{-1}$  was not detectable, indicating that there is no cellulose  $\text{I}_\alpha$  crystalline polymorphism in wheat straw. There is only the absorption band at 710  $\text{cm}^{-1}$ , which indicates that only cellulose  $\text{I}_\beta$  crystalline polymorphism exists in the wheat straw [60].

The  $^{13}\text{C}$ -NMR solution spectrum of unsubstituted cellulose consists of six signals corresponding to the six C-atom of the AGU, as shown in Fig. 5.16, and the corresponding assignment of the signals are listed in Table 5.3. In addition, solid-state Cross-Polarization/Magic Angle Spinning (CP/MAS)  $^{13}\text{C}$ -NMR spectrum of cellulose provides more information, as shown in Fig. 5.17, and the assignment of signals is given in Table 5.4.

A characteristic of CP/MAS  $^{13}\text{C}$ -NMR spectrum of cellulose is the broadening of most signals in  $^{13}\text{C}$ -NMR solution spectrum of cellulose. Sharp signals at about 105, 83–88, and 62–65 ppm stand for the cellulose C-1, C-4, and C-6 carbons, respectively. Differences in the proton spin-lattice relaxation times of the less ordered and the crystalline phase make the signals of C-4 and C-6 carbons separate, which is very useful for the determination of relative crystallinity [91]. The crystallinity (Cr.I.) and amorphicity (Am.I.) indexes are calculated as a percentage

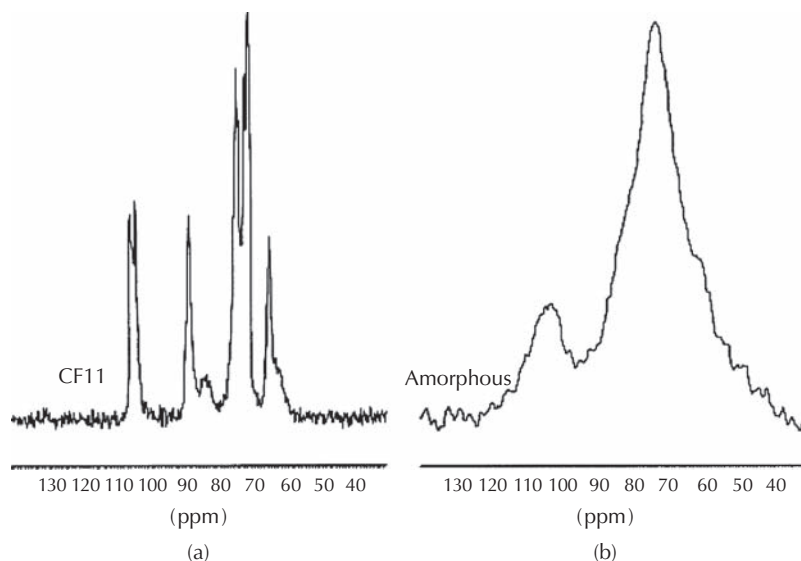


FIGURE 5.17 CP/MAS  $^{13}\text{C}$  NMR spectra of crystalline (a) and amorphous (b) cellulose [63].

**TABLE 5.4** Assignment of CP/MAS  $^{13}\text{C}$ -NMR Signals of Unsubstituted Cellulose

C-atom	Assignment (ppm)
C 1	104.8
C 4 of crystalline cellulose	88.8
C 4 of amorphous cellulose	83.6
C 5	74.7
C 2 and C 3	72.4
C 6 of crystalline cellulose	64.5
C 6 of amorphous cellulose	62.8

of the integrals of the C-4 peaks at 86–92 (a, crystalline cellulose) and 80–86 (b, amorphous cellulose) [63].

$$\text{Cr.I. (\%)} = \frac{a}{a+b} \times 100$$

$$\text{Am.I. (\%)} = 100 - \text{Cr.I.}$$

In addition, the signal of C-1 centered at 104.8 ppm includes the contribution of the crystalline polymorphs  $I_\alpha$  and  $I_\beta$  and their paracrystalline and amorphous components. Digital resolution enhancement could be used to distinguish the crystalline cellulose forms in different cellulose [91].

2D  $^{13}\text{C}$   $^1\text{H}$  NMR spectrum of cellulose has much higher resolution, enabling a larger amount of information to be obtained. This spectrum provides good resolution of signals that overlap in  $^{13}\text{C}$ -NMR and  $^1\text{H}$ -NMR spectra, and more reliable assignments of the signals are possible [92]. Figure 5.18 shows a typical 2D  $^{13}\text{C}$   $^1\text{H}$  NMR spectrum of cellulose.

XRD is another method for the determination of cellulose crystallinity besides FTIR and NMR. Typical XRD curve of cellulose (shown in Fig. 5.19) has strong crystalline peaks at  $14.8^\circ$ ,  $16.8^\circ$ , and  $22.6^\circ$  corresponding to the (110), ( $1\bar{1}0$ ), and (002) planes of crystals, and weak crystalline peaks at  $34.7^\circ$  to the (004) plane [79]. Crystallinity can be calculated from the diffracted intensity data using three different methods [63]. The first one assumes a two-phase structure (crystalline/amorphous) and a line between the intensity minima to obtain an arbitrary background to the diffraction trace, thus, separating an arbitrary crystalline phase from an arbitrary amorphous phase. The degree of crystallinity,  $X_c$ , was calculated by the following formula:

$$X_c = \frac{A_{\text{cr}}}{A_{\text{cr}} + A_{\text{am}}} \times 100,$$

where  $A_{\text{cr}}$  and  $A_{\text{am}}$  are the integrated area of the crystalline and amorphous phases, respectively.

The second approach was the empirical method for native cellulose.

$$\text{Cr.I.} = \frac{I_{002} - I_{\text{am}}}{I_{002}} \times 100,$$

where Cr.I. is the degree of crystallinity,  $I_{002}$  is the maximum intensity of the (002) lattice diffraction, and  $I_{\text{am}}$  is the intensity diffraction at  $18^\circ$ .

In the third deconvolution method, the diffraction profile of the sample was fitted using a Cauchy function, ranging from  $5^\circ$  to  $40^\circ$ , to find the contribution of each individual peak relative to the (110), ( $1\bar{1}0$ ), and (002) and (004) crystallographic planes and the amorphous background. The maximum of the amorphous peak was considered to coincide with the minimum of the diffraction profile of the sample observed between the ( $1\bar{1}0$ ) and (002) peaks.

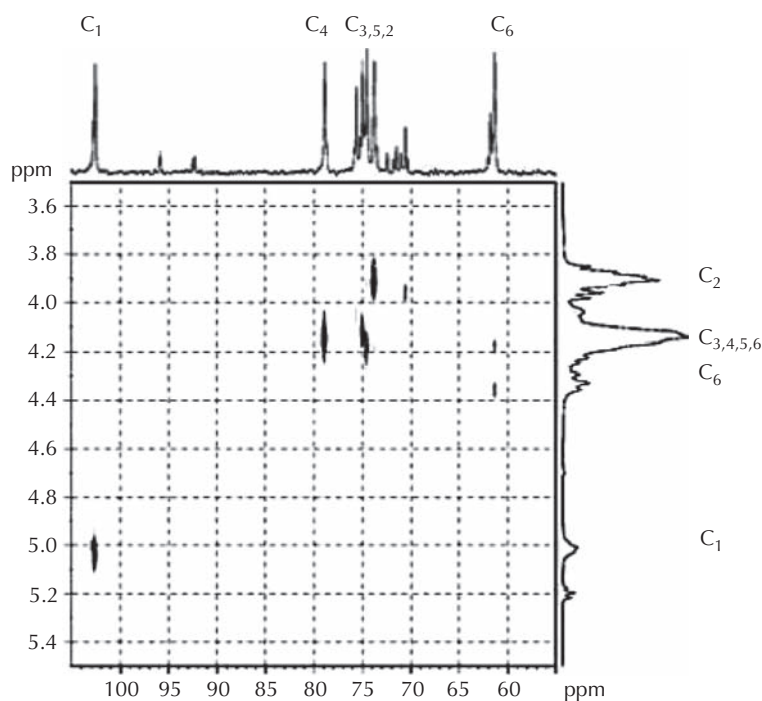


FIGURE 5.18  $^{13}\text{C}$   $^1\text{H}$  NMR spectrum of 5% cellulose dissolved in  $\text{LiCl } 5\text{H}_2\text{O}$  [93].

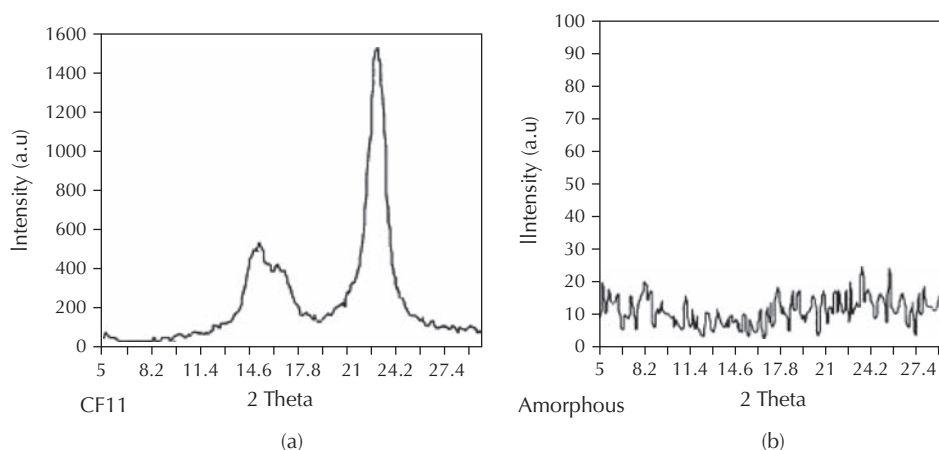


FIGURE 5.19 X-ray diffractograms of crystalline (a) and amorphous (b) cellulose [63].

The degree of crystallinity  $C$  was calculated by the following formula:

$$C = \left[ 1 - \frac{\text{IA}}{\text{IA} + S_p} \right] 100,$$

where IA is the amorphous integrated area, and  $S_p$  is the sum, (101), ( $1\bar{1}0$ ), (002), and (040), of the peak areas.

Among three methods based on FTIR, NMR, and XRD for the determination of cellulose crystallinity, XRD is most reliable. In addition, the crystallite diameter can be determined with XRD based on the width of peaks representing directions perpendicular to the fiber axis ( $110$ ,  $1\bar{1}0$ , and  $200$ ), whereas the crystallite length can be

determined based on the width of peaks representing directions parallel with the fiber axis (004).

### 5.4.2 Dissolution of Cellulose

Due to the stiff molecules and close chain packing via the numerous intermolecular and intramolecular hydrogen bonds, it is extremely hard to dissolve cellulose in water and in most common organic solvents, which constitutes a major obstacle for cellulose application. The efficient dissolution of cellulose is a long-standing goal in cellulose research and development. The solvents for efficient cellulose dissolution have been searched for the



confirmation of cellulose molecules. To date, a number of solvent systems, such as inorganic molten salts like  $\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$  [93], *N*-methylmorpholine-*N*-oxide (NMMO) [94],  $\text{NaOH/urea}$  [95], and ionic liquids (ILs) [96], have been found efficient for cellulose dissolution.

#### 5.4.2.1 Inorganic Molten Salts

Inorganic molten salts can be used as efficient solvents for cellulose in a wide range of DP [97]. The characteristics that mainly determine the dissolution power toward cellulose are recognized as acidity, water content of the melts, and properties of the coordination sphere of the cations. The structures of  $\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$  and  $\text{LiNO}_3 \cdot 3\text{H}_2\text{O}$  are shown in Fig. 5.20. Letters et al. [98] discussed the swelling and dissolution of cellulose in aqueous  $\text{ZnCl}_2$ . They found that swelling occurred in aqueous solution at 55% (w/w) zinc chloride and dissolution at 63% (w/w) zinc chloride. The solubility of cellulose with different DP in  $\text{Ca}(\text{SCN})_2 \cdot 3\text{H}_2\text{O}$  was discussed by Kuga et al. [99]. In the temperature ranging from 120 to 140 °C, they observed the solution of the polymer within 40 min, accompanied by a decrease of DP. Fischer et al. [93] discussed factors that determine the dissolving ability of the melts of  $\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$ ,  $\text{NaSCN/KSCN/LiSCN} \cdot 2\text{H}_2\text{O}$ , and  $\text{LiCl/ZnCl}_2/\text{H}_2\text{O}$  as cellulose solvents. The results showed that besides the specific structure of the molten salt hydrate, the cation and the water content of the melt are the most important factors for the dissolving capability of a molten salt hydrate system.

A multitude of pure molten salt hydrates as well as salt mixtures have been investigated with respect to their interaction with cellulose. It turned out to be reasonable to divide the salt hydrates into groups according to their visible optical effect on cellulose. The classification has been done as follows [93]: molten salts which (a) dissolve, (b) swell, or (c) decompose cellulose or which (d) have no effect on cellulose. Table 5.5 gives this classification for the common molten salt hydrates.

#### 5.4.2.2 *N*-Methylmorpholine-*N*-Oxide

Apart from being widely applied as an oxidant in organic synthesis, *N*-methylmorpholine-*N*-oxide (NMMO) has attracted major interests due to its ability to dissolve cellulose. NMMO is used as direct solvent for cellulose in the commercial Lyocell process as a modern industrial fiber-making technology [94, 100–102]. Main features of Lyocell process are the direct dissolution of cellulose without chemical derivatization and the almost complete recovery of the NMMO. NMMO is able to dissolve cellulose due to the high polarity of its N–O bond, which breaks the hydrogen bond network of the cellulose and forms new hydrogen bonds with the solute [103]. The melting point of NMMO is at 170 °C; when hydrated with one molecule water (NMMO monohydrate; water content 13.3 wt%), its melting point decreases to 74 °C. The melt of NMMO monohydrate at elevated processing temperature of about 100 °C is usually used to dissolve cellulose. Because NMMO is a strong oxidant in Lyocell process, antioxidants such as propyl gallate (PG) are added with NMMO to stabilize the cellulose/NMMO mixture [101]. However, the Lyocell process suffers from the uncontrolled thermal stability of the system NMMO/cellulose/ $\text{H}_2\text{O}$  (a runaway reaction), the high evaporation costs (energy costs), and the high tendency of fibrillation of the Lyocell fiber [102, 104]. At present, NMMO is the only green solvent utilized industrially for cellulose dissolution and regeneration.

#### 5.4.2.3 $\text{NaOH/urea}$ and $\text{LiOH/urea}$

In recent years, Zhang et al. [104, 105] developed a novel solvent system for cellulose based on an  $\text{NaOH/urea}$  aqueous solution precooled to  $-12$  °C, in which the dissolution of cellulose could be achieved rapidly at ambient temperatures (below 20 °C). Interestingly, cellulose with a relatively high molecular weight could not be dissolved in the solvent without being precooled to  $-12$  °C or without urea being added. The addition of urea and the

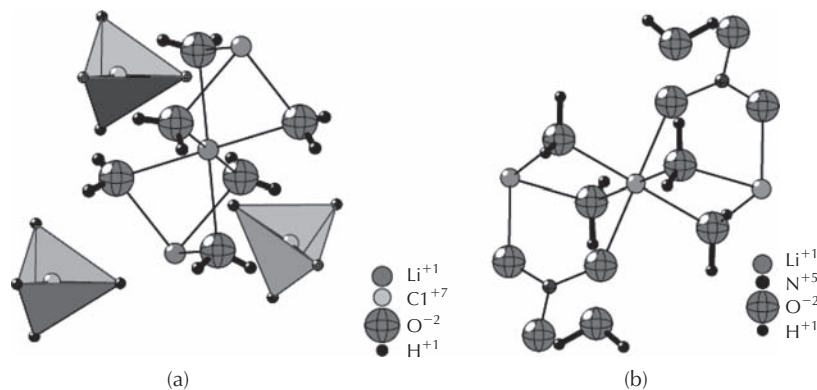
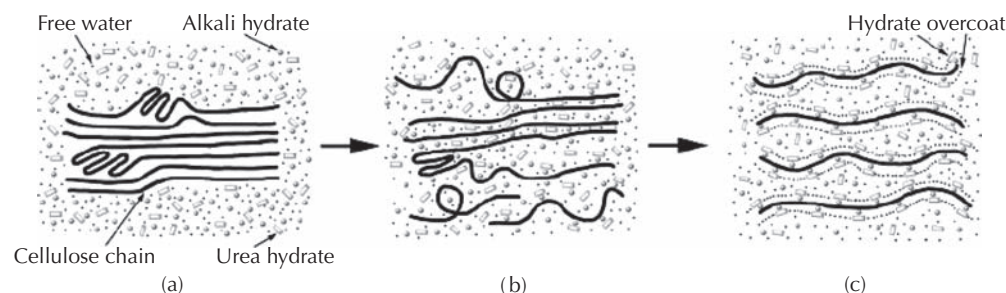


FIGURE 5.20 Crystal structure of  $\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$  (a) and  $\text{LiNO}_3 \cdot 3\text{H}_2\text{O}$  (b).

**TABLE 5.5** Molten Salt Hydrates and Their Interaction with Cellulose<sup>a</sup> [93]

Group	Pure melt	Melt mixtures
Dissolution	$\text{ZnCl}_2 + 3 \text{ 4H}_2\text{O}$ ,	$\text{LiClO}_4 \cdot 3\text{H}_2\text{O} \leq 25\% \text{ Mg}(\text{ClO}_4)_2/\text{H}_2\text{O}$ ,
	$\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$ ,	$\text{LiClO}_4 \cdot 3\text{H}_2\text{O} \leq 10\% \text{ NaClO}_4/\text{H}_2\text{O}$ ,
	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	$\text{LiClO}_4 \cdot 3\text{H}_2\text{O} \text{ MgCl}_2 \cdot 6\text{H}_2\text{O}$ ,
		$\text{NaSCN/KSCN}$ (eutectic) $\text{LiSCN} \cdot 2\text{H}_2\text{O}$ ,
		$\text{LiCl}/2\text{ZnCl}_2/\text{H}_2\text{O}$
Swelling	$\text{LiCl} + 2 \text{ 5H}_2\text{O}$ ,	$\text{LiClO}_4 \cdot 3\text{H}_2\text{O} > 25\% \text{ Mg}(\text{ClO}_4)_2/\text{H}_2\text{O}$ ,
	$\text{LiNO}_3 \cdot 3\text{H}_2\text{O}$ ,	$\text{LiClO}_4 \cdot 3\text{H}_2\text{O} > 10\% \text{ NaClO}_4/\text{H}_2\text{O}$
	$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$	
Decomposition	$\text{Mg}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ ,	$\text{ZnCl}_2/\text{MgCl}_2/\text{H}_2\text{O}$
	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ,	
No effect	$\text{NaOOCCH}_3 \cdot 3\text{H}_2\text{O}$	$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$

<sup>a</sup>Designation of salt-water systems –  $\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$ : the hydrate is solid at room temperature, marked by the symbol ‘:’;  $\text{ZnCl}_2 + 4\text{H}_2\text{O}$ : the hydrate is liquid at room temperature, marked by the symbol ‘+’;  $\text{ZnCl}_2/\text{MgCl}_2/\text{H}_2\text{O}$ : systems of variable compositions, marked by the symbol ‘/’.



**FIGURE 5.21** Schematic dissolution process of the cellulose in LiOH/urea and NaOH/urea aqueous solutions precooled to  $-10^\circ\text{C}$ : (a) cellulose bundle in the solvent, (b) swollen cellulose in the solution, (c) transparent cellulose solution [95].

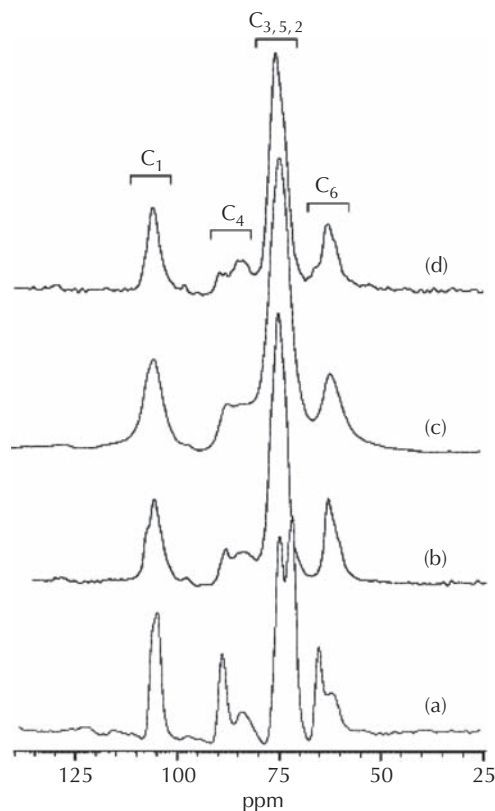
low temperature play an important role in the improvement of the cellulose dissolution because low temperature creates a large inclusion complex associated with cellulose, NaOH, urea, and  $\text{H}_2\text{O}$  clusters, which bring cellulose into aqueous solution, even at relatively high cellulose concentrations [106]. Moreover, the cellulose dope could remain in a liquid state for a long period at about  $0$  to  $5^\circ\text{C}$ . Cai et al [107] also found that LiOH/urea solution is a direct solvent for cellulose. Cellulose could be dissolved rapidly in 4.6 wt% LiOH/15 wt% urea aqueous solution and precooled to  $-10^\circ\text{C}$  to create a colorless transparent solution. The results revealed that the cellulose existed as semi-stiff-chains in the LiOH/urea aqueous solution.

Cai et al. [95, 106] systematically studied rapid dissolution of cellulose in LiOH/urea and NaOH/urea aqueous solutions. The schematic dissolution process of the cellulose is shown in Fig. 5.21. They found that the dissolution power of the solvent systems was in the order of LiOH/urea > NaOH/urea >> KOH/urea aqueous solution. Cellulose having viscosity-average molecular weight ( $\overline{M}_\eta$ )

of  $11.4 \times 10^4$  and  $37.2 \times 10^4$  could be dissolved, respectively, in 7% NaOH/12% urea and 4.2% LiOH/12% urea aqueous solutions precooled to  $-10^\circ\text{C}$  within 2 min, whereas both of them could not be dissolved in KOH/urea aqueous solution. The CP/MAS  $^{13}\text{C}$ -NMR spectra of native and regenerated cellulose are shown in Fig. 5.22. It was indicated that LiOH/urea and NaOH/urea aqueous solutions as nonderivatizing solvents broke the intra- and intermolecular hydrogen bonding of cellulose and prevented the approach toward each other of the cellulose molecules, leading to the good dispersion of cellulose to form an actual solution. Dissolution of cellulose in NaOH/urea and its utilization have received much attention around all over the world up to date.

#### 5.4.2.4 Ionic Liquids and Ionic Liquid Analogues

The first example of the cellulose dissolution and processing using ILs might be dated back to 1934. In a patent,

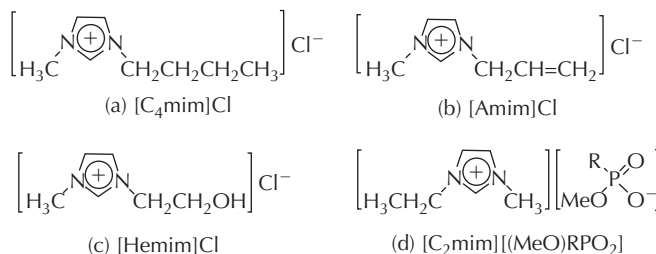


**FIGURE 5.22** CP/MAS  $^{13}\text{C}$  NMR spectra of the native cellulose (a) and the cellulose recovered from LiUr (b), NaUr (c), and KUr (d) [95].

Graenacher [108] discovered that molten *N*-ethylpyridinium chloride, in the presence of nitrogen-containing bases, could dissolve cellulose. Unfortunately, this did not attract significant attentions due to the lack of knowledge on this kind of substances at that time and relatively high melting points of the pyridinium salts, 118–120 °C. Later, it has been shown that the melting point can be lowered to 77 °C by mixing the IL with 50% *N,N*-dimethylformamide (DMF) or dimethylsulfoxide (DMSO); the mixtures thus obtained dissolve cellulose [109]. In 2002, Swatloski et al. [96] reported that some alkyl substituted imidazolium ILs with different anions could be used to dissolve and process cellulose with a high DP up to 1200 without degradation. Among the ILs they studied, 1-butyl-3-methylimidazolium chloride ([C<sub>4</sub>mim]Cl, Fig. 5.23a) exhibited the best

dissolving capability, and up to 10 wt% cellulose solution could be obtained in this IL by heating. Cellulose could also be dissolved in 1-butyl-3-methylimidazolium bromide ([C<sub>4</sub>mim]Br) and 1-butyl-3-methylimidazolium sulfocyanate ([C<sub>4</sub>mim]SCN) but with less than half solubility in [C<sub>4</sub>mim]Cl. However, ILs containing “noncoordinating” anions, including [BF<sub>4</sub>]<sup>−</sup> and [PF<sub>6</sub>]<sup>−</sup>, were found to be nonsolvents for cellulose. In the case of alkylimidazolium chlorides with cations from [C<sub>4</sub>mim]<sup>+</sup> to [C<sub>8</sub>mim]<sup>+</sup>, their solubility of cellulose decreased with increasing length of alkyl group substituted on the imidazolium ring.

This finding has been providing a new and versatile platform for the wide utilization of cellulose resources and creation of novel functional materials. Since then, the dissolution of cellulose in ILs and its application have attracted an increasing attention. However, relatively high dissolution temperatures (often above 80 °C) are often required for dissolving cellulose; this possibly results in the thermal decomposition of room-temperature ionic liquid (RTILs) [110] and produces some organohalogenides [111], which have uncertain toxicity and hazardousness to zoology and ecosystems after ineluctable release into the environment. Many kinds of cation- or anion-functionalized ILs, especially RTIL, with lower melting point and toxicity and higher solubility for cellulose were reported. By appending the allyl (−CH<sub>2</sub>CH=CH<sub>2</sub>) moiety on the imidazole cation, Zhang and Ren [90, 112] synthesized a novel cation-functionalized RTIL, 1-allyl-3-methylimidazolium chloride (AmimCl, Fig. 5.23b), which has outstanding capability for dissolving cellulose. This RTIL is a nonderivatizing solvent with higher solubility for cellulose than [C<sub>4</sub>mim]Cl. Solution containing 5 wt% cellulose in AmimCl could be formed within only 15 min at 100 °C without any pretreatment or activation. In addition, it is more convenient for the application of AmimCl than [C<sub>4</sub>mim]Cl because this IL is liquid at room temperature. AmimCl shows a lower melting point at *ca.* 17 °C and a considerably lower viscosity of 685 MPa·s at 30 °C, whereas [C<sub>4</sub>mim]Cl has a melting point at 65 °C and viscosity of 11 000 MPa·s at 30 °C [2]. The lower melting point and viscosity of AmimCl are attributed to its suppressed crystallization by an allyl group on the *N*-position [113]. In 2005, another cation-functionalized RTIL, 1-(2-hydroxyethyl)-3-methylimidazolium chloride (HemimCl, Fig. 5.23c), was reported as the



**FIGURE 5.23** Chemical structure of ionic liquids for cellulose dissolution.

nonderivatizing solvent for cellulose by Luo et al. [114]. This IL introducing hydroxyl groups on the imidazole cation has relatively high solubility for cellulose. Solutions containing 5% cellulose in HemimCl could be formed at 60 °C and 6.8% at 70 °C. However, the relatively low thermal stability of HemimCl limits its application.

The dissolution of cellulose can also be achieved by introducing functionalization on anions. An acetate RTIL, 1-ethyl-3-methylimidazolium acetate (EmimAc), was found to be a good cellulose solvent [115]. In this solvent, two kinds of ions,  $[\text{Emim}]^+$  and  $\text{CH}_3\text{COO}^-$ , are in a free state in solution, and both of them could interact with hydroxyls of cellulose and generate complex  $[\text{Emim}]\text{-Cellulose-Ac}$ , resulting in the break of both intermolecular and intramolecular hydrogen bonding. It shows a much higher capability for cellulose dissolution besides its very low melting point ( $<-20$  °C) and viscosity ( $\sim 140$  cPs at 25 °C). Recent studies showed that the preparation time for a 10 wt% cellulose spinning dope was about 45–60 min [116], and a cellulose concentration as high as 20 wt% in EmimAc could be reached [117]. Moreover, EmimAc was considered to be less toxic and corrosive than chlorides and even biodegradable [118]. These features make EmimAc a promising solvent for the processing and homogeneous derivatization of cellulose. Fukaya et al. [119] found that a series of formate RTILs exhibited superior solubility for a wide range of polysaccharides including cellulose. These RTILs have significantly lower viscosities than previously reported halogenated imidazolium RTILs, for example 1-allyl-3-methylimidazolium formate has viscosity of 66 cP at 25 °C and 1-allyl-3-ethylimidazolium formate has viscosity of 67 cP at 25 °C. Compared with RTIL AmimCl, 1-allyl-3-methylimidazolium formate dissolved cellulose at lower temperatures and reached larger concentrations. In 2008, Fukaya et al. [120] reported that a series of anion-functionalized RTILs, alkylimidazolium salts containing dimethyl phosphate, methyl methylphosphonate, or methyl

phosphonate [121], had the potential to solubilize cellulose under mild conditions. Especially, 1-ethyl-3-methylimidazolium methylphosphonate (Fig. 5.23d) could enable the preparation of 10 wt% cellulose solution at 45 °C within 30 min and 2–4 wt% cellulose solution even at room temperature without any pretreatment and heating. These functionalized ILs exhibit higher solubility for cellulose, and it is more convenient for processing and application.

Heinze et al. [122] examined the dissolving capability of different RTILs such as  $[\text{C}_4\text{mim}]\text{Cl}$ , 3-methyl-*N*-butylpyridinium chloride, and benzyldimethyl(tetradecyl) ammonium chloride for cellulose.  $[\text{C}_4\text{mim}]\text{Cl}$  was the most appropriate cellulose nonderivatizing solvent because it has strong ability to dissolve cellulose with DP in the range from 290 to 1200 up to very high concentrations and almost no degradation of cellulose after the dissolution. In their experiments,  $[\text{C}_4\text{mim}]\text{Cl}$  could dissolve the cotton linter (DP = 1198) with the concentration of 10 wt%, and DP only slightly decreased to 812.  $^{13}\text{C}$ -NMR spectrum of cellulose in  $[\text{C}_4\text{mim}]\text{Cl}$  is shown in Fig. 5.24. Erdmenger et al. [123] investigated the influence of the length of alkyl chain from  $\text{C}_2$  to  $\text{C}_{10}$  of 1-alkyl-3-methylimidazolium chlorides on the solubility of cellulose at 100 °C. The results showed that cellulose was easily dissolved in 1-alkyl-3-methylimidazolium-based ILs with even-numbered alkyl chains compared with odd-numbered alkyl chains with below six carbon units.  $[\text{C}_4\text{mim}]\text{Cl}$  was found to be efficient for the even-numbered alkyl chains, dissolving 20 wt% of cellulose, whereas 1-heptyl-3-methylimidazolium chloride was the most efficient odd-numbered ionic liquid, dissolving 5 wt% of cellulose. The reason for this odd-even effect and the large difference in optimal chain length for the cellulose dissolution is still not understood.

The dissolution of cellulose in RTILs can be significantly enhanced by using energy irradiation, such as ultrasound and microwave. Swatoski et al. [96] found that  $[\text{C}_4\text{mim}]\text{Cl}$  could dissolve up to 25 wt% of cellulose

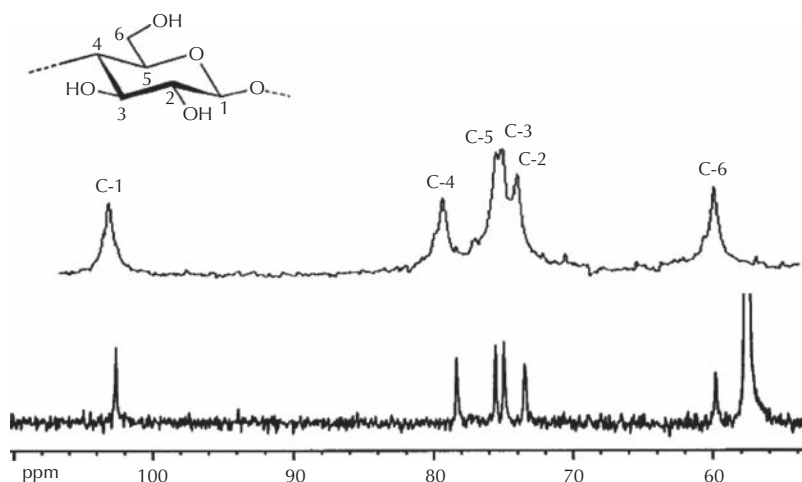


FIGURE 5.24  $^{13}\text{C}$  NMR spectra of cellulose dissolved in the IL  $[\text{C}_4\text{mim}]\text{Cl}$  (top) and in DMSO/TBAF (bottom) [122].



under heating supported by short microwave pulses, whereas it could dissolve only a 10 wt% of cellulose under conventional heating. Mikkola et al. [124] also proved that the dissolution process of cellulose in ILs  $[C_4mim]Cl$  and AmimCl could be considerably enhanced by means of high-intensity ultrasound. In comparison with conventional heating, the dissolution time decreased profoundly. For example, in order to obtain a 5 wt% cellulose solution in AmimCl, 1 h (or even more) was needed using conventional heating, whereas only 2 min was need using ultrasound irradiation. A solution containing up to 27% of microcrystalline cellulose was easily obtained in AmimCl under periodic ultrasound pulses (1 min of sonication followed by a 1 min pause).

Dissolution of cellulose in ILs was attributed to their ability to break the extensive network of hydrogen bonds existing in cellulose. In the case of alkylimidazolium chloride, the high chloride concentration in the RTIL enhanced its ability to dissolve cellulose. It is speculated that the high chloride ion concentration breaks the extensive hydrogen bonding of cellulose, enabling dissolution even at relatively high concentration [96]. Although some studies [125, 126] suggested that little or weak interaction between cations of IL and the glucose of cellulose existed, Zhang et al. [90] believed that cations of IL were involved in the dissolution process, and thus, their role in the dissolution mechanism should not be neglected. A possible mechanism of cellulose dissolution in AmimCl is shown in Fig. 5.25. Insight into the mechanism of dissolution of cellulose in ILs was also achieved by applying NMR spectroscopy [122, 127].  $^{13}C$ -NMR signals of cellulose dissolved in  $[C_4mim]Cl$  were recorded at 80 °C, and the spectrum obtained was compared with that of the same cellulose in a typical nonderivatizing solvent system, DMSO/TBAF. The similarity of the chemical shifts of the AGU carbon atoms indicated that the IL is a nonderivatizing solvent for cellulose. The spectra also indicated that cellulose oligomers are disordered in the IL/DMSO solution. This result was similar to that observed for aqueous solutions, despite the considerable differences of the two media.

The development of ILs based on a combination of the inexpensive and readily available components choline chloride ( $ChCl$ ;  $HOCH_2CH_2N(Me)_3Cl$ ) and zinc chloride has also been reported as cellulose solvents [128–130]. Moreover, Abbott et al. [131, 132] developed a novel green solvent for cellulose dissolution, a eutectic mixture of a

range of quaternary ammonium salts, such as choline chloride, and urea, which forms an ionic liquid analogue with the advantage of being nontoxic and readily biodegradable but has no Lewis acidity.

#### 5.4.2.5 Other Cellulose Solvents

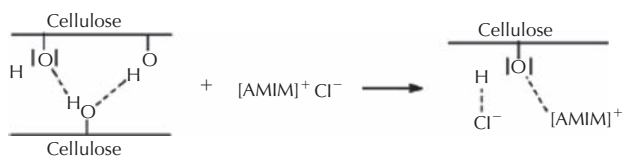
There are still a limited number of solvent systems used for cellulose dissolution, including DMAc/LiCl [133], DMF/ $N_2O_4$  [134], DMSO/TBAF [135], etc. Heinze et al. [136] dissolved cellulose with a DP up to 650 in DMSO/TBAF (10–20%, w/v) without any pretreatment within 15 min at room temperature.  $^{13}C$ -NMR analysis showed that this solvent could be classified as a nonderivatizing solvent. Hattori et al. [85] found that the cellulose dissolution in the ethylenediamine/sodium thiocyanate (EDA/NaSCN, 54/46, w/w) could take place at room temperature, and the maximum solubility achieved was 16% (w/w) for cellulose that has a DP of 210. The cellulose dissolved was stable for 30 days when stored at room temperature. The authors believed that this solvent system had high potential for cellulosic fiber and film formations.

### 5.4.3 Regeneration of Cellulose

Regenerated cellulose has been used as a material for many centuries, mainly in the form of the fibers or in the form of films, powders, beads, and membranes. The dissolution of cellulose in a series of novel green solvents has been providing a new platform for regenerated cellulose applications. Cellulose can be precipitated from the solution by the addition of antisolvents. After dissolution and regeneration, cellulose morphology is significantly changed and a relatively homogeneous macrostructure is obtained. The physicochemical properties of regenerated cellulose are affected by the dissolution and regeneration processes. Different structural forms of regenerated cellulose such as fibers, films, powders, beads, and membranes can be prepared by changing the processing conditions, especially regeneration conditions.

Traditional viscose route to regenerate cellulose is often slow and environmentally unfriendly. Nowadays, searching for new spinning systems with nonpollution process is a key for advancement of the cellulose industries. At present, a number of nonderivatizing and derivatizing solvents of cellulose have been investigated [95, 137]. The nonderivatizing solvents include aqueous inorganic complexes (cuoxam, cadoxen, cuen), 10% NaOH aqueous solution, mineral acids, melts of inorganic salt hydrates, DMAc/LiCl, DMSO/TEA/ $SO_2$ ,  $NH_3/NH_4SCN$ , NMMO, NaOH/urea, and ILs. The derivatizing solvents mainly are  $CF_3COOH$ ,  $HCOOH$ , DMF/ $N_2O_4$ , and DMSO/paraformaldehyde.

It is noted that NMMO is a good solvent of cellulose and has made its technical breakthrough, leading to a new class of



**FIGURE 5.25** Possible dissolution mechanism of cellulose in AmimCl [90].



man-made cellulose fibers with the generic name Lyocell [95, 102, 137, 138]. Lyocell process is an environmentally friendly process of cellulose-fiber spinning using a direct solvent system, NMMO. Regenerated cellulose fibers from rice straws with a diameter of 10 to 25  $\mu\text{m}$  and initial modulus of 11 to 13 GPa were prepared by wet spinning in rice straw/MMNO solution [139, 140]. XRD analysis indicated that the regenerated rice straw fibers were classified as cellulose II. This observation indicated a potential utilization of rice straw as an alternative to wood pulp as a cellulose-based fiber material. However, the uncontrolled thermal stability of the system NMMO/cellulose/ $\text{H}_2\text{O}$ , the high evaporation costs (energy costs), and the high tendency of fibrillation of the Lyocell fiber are the disadvantages of the Lyocell process [102, 104].

Cellulose fibers regenerated from other cellulose solvents were also investigated. Araki et al. [133] prepared polyrotaxane/cellulose blends fibers by wet-spinning of the polyrotaxane/cellulose blend solution dissolved in solvent system DMAc/LiCl into methanol and a subsequent annealing. The results showed that the Young's modulus and tensile strength of the fibers with polyrotaxane/cellulose ratio of 1:1 and 2:1 were higher than those of pure cellulose fiber.

Cai et al. [104] first attempted to prepare the wet-spinning of cellulose multifilaments from the cellulose solution based on NaOH/urea aqueous solution using a pilot machine. From the cellulose dope formed after dissolution of cellulose in 7 wt% NaOH and 12 wt% urea aqueous solution precooled to  $-12^\circ\text{C}$ , high-quality cellulose multifilaments were spun successfully using a pilot machine. The cellulose crystals were completely transformed from cellulose I to II during these processes. The multifilament fibers had a circular cross-sectional shape and a smooth surface as well as good mechanical properties. The multifilament fibers produced from the NaOH/urea solvent system are shown in Fig. 5.26. The CP/MAS  $^{13}\text{C}$ -MR spectra and SEM images of filaments are shown in Figs. 5.27 and 5.28, respectively.

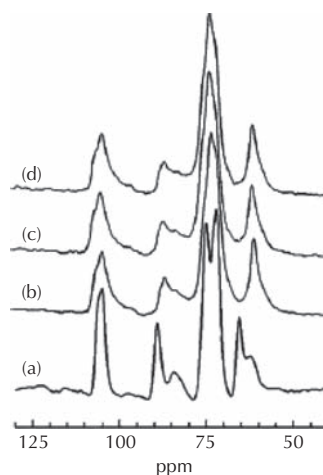
The dissolution of cellulose in novel green solvents, ILs, has been providing a new platform for regenerated cellulose preparation. Cellulose could be regenerated from the IL solution by the addition of antisolvents such as water, ethanol, acetone, and isopropanol. Different structural forms of regenerated cellulose such as fibers, films, powders, beads, and membranes can be prepared by changing processing conditions, especially regeneration conditions [141]. Rogers and his coworkers [96, 141–145] carried out comprehensive studies on cellulose dissolution in ILs and its regeneration to create advanced cellulose-based materials. Because of his great contribution, Rogers has become a winner of the 2005 US Presidential Green Chemistry Challenge Awards, indicating the importance of this work in society. It was reported that Ionicell fiber, the cellulose fiber obtained from ILs such as  $[\text{C}_4\text{mim}]\text{Cl}$  by

dissolution and regeneration, has good properties similar to those of Lyocell fiber. Kosan et al. [117] also successfully prepared cellulose dopes using ILs, which could be shaped by a dry-wet spinning process to manufacture cellulose fibers. Ionicell fiber would be a new kind of environmentally friendly promising fiber following the Lyocell fiber.

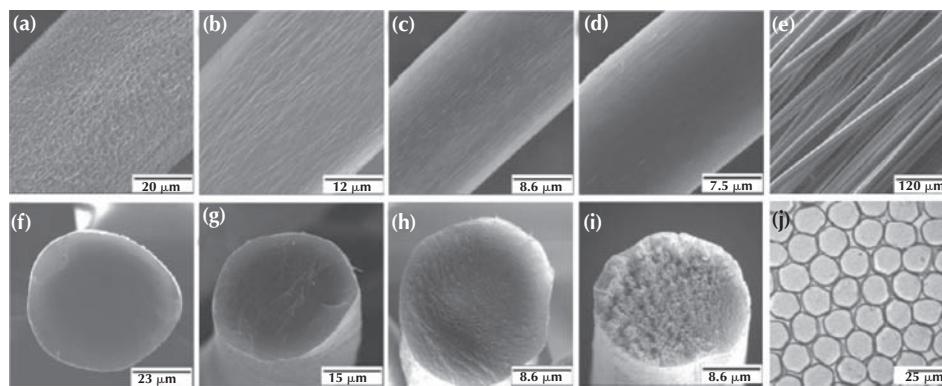
Cellulose blended or composite materials can be prepared when the cellulose solution contains other additives, e.g., dyes and metal complexing agents. The incorporated functional additives can be dissolved or dispersed in solvents before and after dissolution of the cellulose. With this simple approach, many kinds of cellulose composites with different structural forms can be



**FIGURE 5.26** Photograph of multifilament fibers produced from cellulose dope using industrial (front) and analytical (back) grades of NaOH and urea [104].



**FIGURE 5.27** CP/MAS  $^{13}\text{C}$  MR spectra of cellulose (a) and filaments on the spinning process at each stage: U 2 I (b), U 2 II (c), and U 2 III (d). The multifilament fibers on the spinning process at each stage of roller I, II, and III, respectively, were coded as U 2 I, U 2 II, and U 2 III [104].



**FIGURE 5.28** Scanning electron microscopic images of the surface (top) and cross section (bottom) of the U 2 filaments at each stage from the Nelson type roller I (a) to the take up device (d). A bundle of filaments on the take up device was observed by using SEM (e) and by using optical microscopy (j) [104].

easily obtained [146]. Turner et al. [141] proposed a new method for introducing enzymes into cellulosic matrixes, which can be formed into membranes, films, or beads using a cellulose-in-IL-dissolution and regeneration process. Xie et al. [147] developed a method to prepare wool keratin/cellulose blended materials by the dissolution and regeneration of wool keratin fibers in  $[C_4mim]Cl$ . Egorov et al. [148] prepared cellulose films containing entrapped analytical reagents suitable for metal-ion detection by joint dissolution of the cellulose and the reagents in IL followed by precipitation with water. This method provided a new technology for quantitative determination of transition metal cations, e.g., colorimetric determination of divalent zinc, manganese, and nickel with detection limits at the  $10^{-6} \text{ mol} \cdot \text{L}^{-1}$  level. Kadokawa et al. [149–151] reported a facile method for the preparation of composites composed of cellulose and a polystyrene-type polymeric IL using an imidazolium-type polymerizable IL. Cellulose-nanohydroxyapatite composite scaffolds with high and open porosity were successfully prepared by poly(methyl methacrylate) particulate leaching with  $[C_4mim]Cl$  as cellulose solvent [152, 153]. Swatloski et al. [142] prepared magnetic cellulose materials in ILs. It can be indicated from XRD data that the magnetic materials were wrapped into the 25 nm particles and their chemical properties were not changed. The preparation of cellulose blended or composite materials using ILs would broaden the scope of the application of conventional cellulose.

#### 5.4.4 Hydrolysis of Cellulose

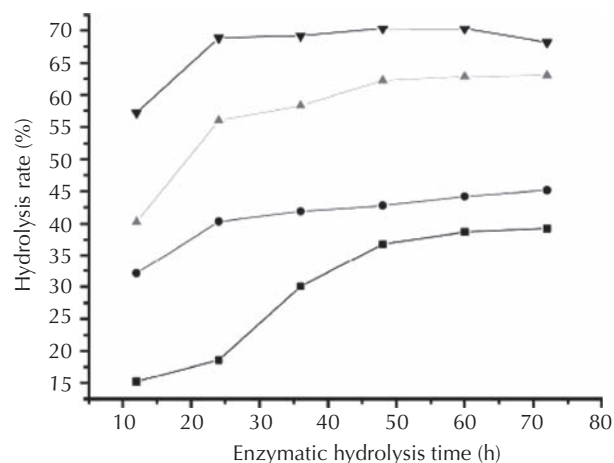
Biofuel such as bioethanol is one of the most promising applications of straw cellulose in industries in the future. For the production of bioethanol, cellulose ultimately should be hydrolyzed to glucose for fermentation. Hydrolysis of cellulose to fermentable glucose in aqueous media catalyzed by the cellulase or acid has the disadvantage of

slow reaction rates in industry due in large part to the highly crystalline structure of cellulose and inaccessibility of cellulase or acid adsorption sites. To make the hydrolysis process viable for producing simple sugars for fermentation to produce ethanol fuel and other bio-based products, this highly ordered structure of cellulose should be overcome. Pretreatment methods, which increase the surface area accessible to water and cellulases, are vital for improving the hydrolysis and conversion of cellulose to glucose.

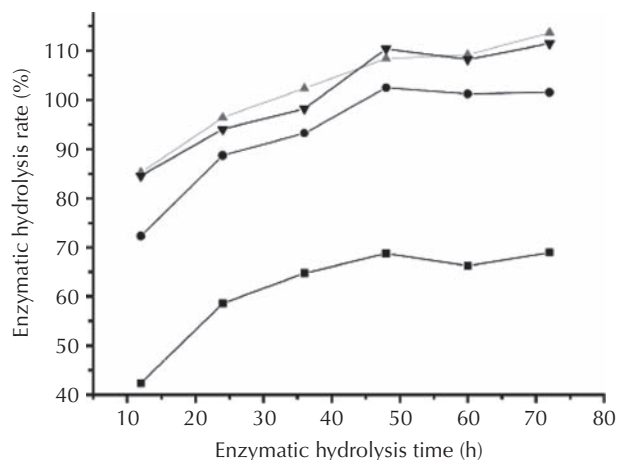
In a novel technique, the microcrystalline cellulose was first subjected to a dissolution treatment and then recovered as essentially amorphous or as a mixture of amorphous and partially crystalline cellulose by rapidly quenching the solution with an antisolvent. Dadi et al. [154, 155] made an attempt to disrupt the cellulose structure in  $[C_4mim]Cl$  and AmimCl. The results indicated that the initial enzymatic hydrolysis rates for IL-treated cellulose were up to 90 times greater than those of untreated cellulose, which was because of the conversion of crystalline cellulose to amorphous cellulose during pretreatment with IL. Zhao et al. [156] conducted a systematic study on the cellulose regeneration and the subsequent enzymatic hydrolysis from a number of chloride- and acetate-based ILs. The study confirmed that all regenerated celluloses were less crystalline (58–75% lower) and more accessible to cellulase (>2 times) than untreated substrates. As a result, regenerated celluloses were hydrolyzed 2–10 times faster than the respective untreated celluloses. A complete hydrolysis of cellulose could be achieved in 6 h given the *Trichoderma reesei* cellulase/substrate ratio (w/w) of 3:20 at 50 °C. A thorough removal of IL residues after cellulose regeneration is highly recommended because of cellulase inactivation in the presence of ILs. NMMO monohydrate was used by Kuo et al. [103] in the pretreatment for enzymatic hydrolysis enhancement of SCB cellulose. Bagasse of 20 wt% was readily dissolved in NMMO monohydrate at 130 °C within 1 h. After dissolution, bagasse was regenerated by rapid precipitation with water as a porous and amorphous mixture

of its original components. The regenerated bagasse significantly enhanced the enzymatic hydrolysis. Not only the releasing rate of reducing sugars but also the hydrolysis yield were enhanced at least two-fold as compared with that of untreated bagasse. The cellulose fraction of regenerated bagasse was nearly hydrolyzed to glucose after 72 h hydrolysis with Cellulase AP3. The recycled NMMO demonstrated the same performance as the fresh one on bagasse pretreatment for hydrolysis enhancement. The regenerated bagasse was directly used in simultaneous saccharification and fermentation (SSF) for ethanol production by *Zymomonas mobilis*. No negative effect on ethanol fermentation was observed, and ethanol yield approximately 0.15 g ethanol/g bagasse was achieved. Liu and Chen [89] used  $[C_4mim]Cl$  to treat wheat straw and steam-exploded wheat straw (SEWS) to improve the enzymatic hydrolysis rates. The enzymatic hydrolysis results showed that the hydrolysis rates of materials treated with  $[C_4mim]Cl$  were improved, as shown in Figs. 5.29 and 5.30. The hydrolysis rate of treated wheat straw could reach 70.37% and the SEWS could be completely hydrolyzed, whereas hydrolysis rates of the wheat straw and SEWS treated with water (the control) were 42.78 and 68.78% under the same conditions, respectively. The FTIR analysis and DP measurement indicated that the hydrolysis rates improvement was attributed to the decrease of the polymerization degrees of cellulose and hemicelluloses, the absolute crystallinity degree of cellulose, and the increase of its reaction accessibility.

To simplify the entire process, enzymatic or acidic in situ saccharification processes that eliminate the need to recover regenerated cellulose were also reported. Kamiya et al. [157] established an enzymatic saccharification of cellulose in an imidazolium type IL with an alkylphosphate anion, which will be very useful in integrated bioprocesses such as bioethanol production from straws. A novel method



**FIGURE 5.29** Enzymatic hydrolysis rates of wheat straw treated with  $[C_4mim]Cl$  [89] ■, 10 min with water; ●, 2 min with  $[C_4mim]Cl$ ; ▲, 6 min with  $[C_4mim]Cl$ ; ▼, 10 min with  $[C_4mim]Cl$ .



**FIGURE 5.30** Enzymatic hydrolysis rates of steam exploded wheat straw treated with  $[C_4mim]Cl$  [89] ■, 10 min with water; ●, 2 min with  $[C_4mim]Cl$ ; ▲, 6 min with  $[C_4mim]Cl$ ; ▼, 10 min with  $[C_4mim]Cl$ .

for the hydrolysis of cellulose from corn stalk, rice straw, pine wood, and bagasse catalyzed by mineral acids in  $[C_4mim]Cl$  was developed by Li et al. [158, 159]. This method could facilitate the hydrolysis of cellulose with dramatically accelerated reaction rates at 100 °C under atmospheric pressure and without pretreatment.

## 5.5 CHEMICAL MODIFICATION AND ITS UTILIZATION

Cellulose is used for many centuries not only as a construction material, mainly in the form of intact wood, the natural textile fibers, or in the form of paper and board, but also as a versatile starting material for subsequent chemical modification to a variety of cellulose derivatives (ethers and esters) to be used in the form of fibers, films, food casings, membranes, and sponges in many areas of industries and life [2].

Effective utilization of straw cellulose not only reduces the consumption of the limited fossil resources but also protects the environment of the Earth. Due to the three hydroxyl groups available for modification within one AGU and the polymeric character of the cellulose, a great variety of chemical modifications, such as esterification with linear and cyclic anhydrides, etherification including carboxymethylation and quaternization, tosylation, tritylation, and carbanilation are possible [88].

In recent years, chemical modification of cellulose has gained much attention, especially in homogeneous reaction media with the development of novel green solvents for cellulose. For the homogeneous cellulose derivatization reaction, suitable solvent system that can dissolve cellulose and provide a feasible reaction environment is prerequisite. Several direct solvents for cellulose have been developed over the past, and a few of them are suitable for the

chemical functionalization of the cellulose. These solvents include DMAc/LiCl [160–162], DMSO/TBAF [136, 163], some molten salt hydrate, such as  $\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$  and  $\text{LiSCN} \cdot 2\text{H}_2\text{O}$  [93, 164], and ILs.

### 5.5.1 Acetylation

In recent years, there has been strong emphasis to develop new cellulose-based materials because of the biodegradability and renewable aspects of these materials [165]. Acetylation of cellulose with acetyl chloride or acetic anhydride has been known for a long time. Cellulose acetate (as shown in Figs. 5.31 and 5.32) is one of the most commercially important cellulose derivatives. It is widely used in textiles because of its low cost, toughness, gloss, high transparency, natural feel, and other favorable aesthetic properties. Cellulose acetate fibers in cigarette filters are designed to absorb vapors and accumulate particulate smoke components. Cellulose acetate is also used as a carrier for photographic negatives, motion picture film (celluloid), microfilm, microfiche, and audio tape [165–167]. In addition, the acetylation of cellulose is also widely used for the protection of hydroxy groups and the purification and structural elucidation of natural products. The often-used cellulose acetates include diacetates having an average degree of substitution (DS) in the range from 2.2 to 2.7 and triacetates having an average DS above 2.8 [137]. Various methods have been developed for producing cellulose acetates, in which acetic anhydride and acetyl chloride are commonly used as acetylating reagents. Industrially, cellulose acetates are often produced by reaction of cellulose with an excess of acetic anhydride in the presence of sulfuric acid or perchloric acid as the catalysts [168]. Biswas et al. [169] acetylated the cellulose from agricultural residues with acetic anhydride and catalytic amount of sulfuric acid to make cellulose acetate, following hydrolyzation with dilute acid at a moderate temperature to remove hemicelluloses. The results showed that the pretreatment used to hydrolyze the hemicelluloses was also useful for cellulose acetate production. Compared with 0.5, 1.8, and 13.5 wt% of conversion rate, respectively, for wheat straw, corn fiber, and rice hulls without pretreatment, the conversion rate increased to about 25 wt% after pretreatment.

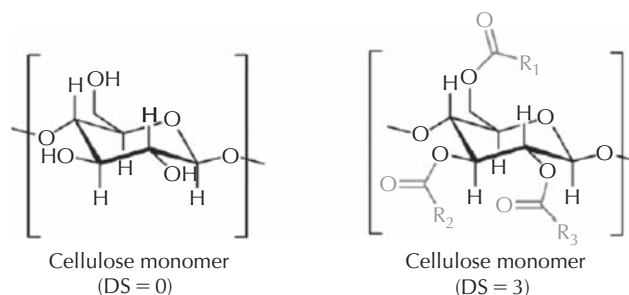


FIGURE 5.32 Structure of a cellulose monomer and related cellulose triacetate [170].

Biswas et al. [165, 167] developed a process for esterification of cellulose with acetic anhydride in the presence of a novel catalytic amount of iodine to produce cellulose triacetate, and the acetylation reaction is shown in Fig. 5.33. The results showed that the acetic anhydride in the presence of a catalytic amount of iodine was an excellent acylating reagent for cellulose. The reaction time required for complete esterification at room temperature was 12 h, whereas it was reduced to 10 min when the reaction was conducted at 100 °C. Iodine activates the carbonyl group of acetic anhydride making the latter more reactive, as shown in Fig. 5.34. This acetic anhydride iodine combination works only in the absence of a solvent. When cellulose was heated with a mixture of acetic acid and iodine instead of acetic anhydride, no acetylation occurred.

Due to the heterogeneous nature of the aforementioned reaction, it is impossible to synthesize partially substituted cellulose acetates directly. Homogeneous derivatization of cellulose has three main advantages over the heterogeneous path [137, 171]. First, the DS of the cellulose derivative can be effectively controlled by adjusting reaction conditions, such as the reaction time, the temperature, and the molar ratio of derivatizing agent to cellulose. Second, the substituent groups are introduced regularly along the cellulose backbone. Third, the physicochemical properties of products thus obtained are controlled much better than those prepared under heterogeneous conditions.

Several molten inorganic salt hydrates were applied as media for the homogeneous acetylation of cellulose. The acetylation in molten thiocyanate leads to the formation of amorphous cellulose acetate. The formation of cellulose

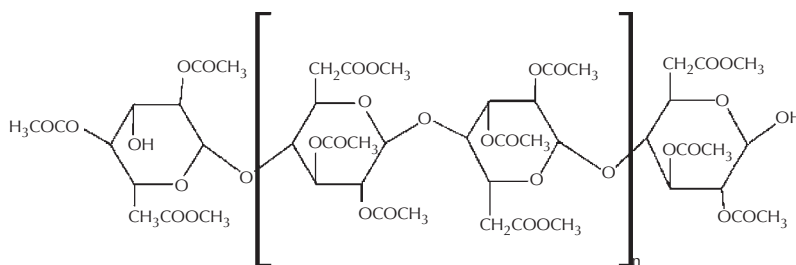


FIGURE 5.31 Structure of cellulose triacetate [169].



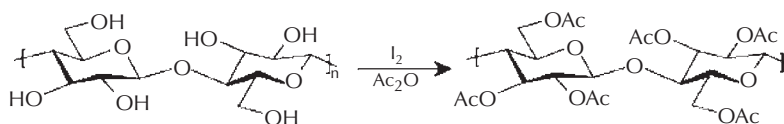


FIGURE 5.33 Conversion of cellulose to cellulose triacetate [167].

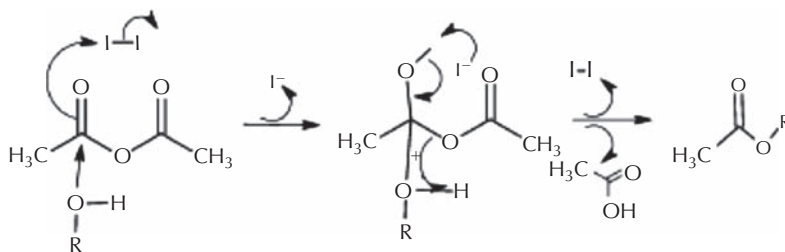


FIGURE 5.34 Mechanism of iodine catalyzed acetylation [165].

acetate depended on the used molten salt hydrate itself as well as on the water content of the melt. However, the only melt in which the acetylation was successful was the eutectic mixture of NaSCN and KSCN with addition of 10% LiSCN·2H<sub>2</sub>O. The most important condition for the success of the reaction was the minimization of the water content of the melt. The acetylation was carried out at 130 °C with a high excess of acetic anhydride (50–100%). During a short reaction time (0.5–3 h), cellulose acetate with a DS in the range between 1 and 2.5 were obtained [93].

Cellulose acetylation under homogeneous reaction conditions, e.g., in LiCl/DMAc, includes three steps [160], namely activation, dissolution, and subsequent reaction with the derivatizing agent. Activation can be carried out by solvent exchange, e.g., treatment with water, methanol, and DMAc, by distillation of part of the solvent and by heating native cellulose under reduced pressure. Solubilized cellulose is then derivatized, e.g., by reaction with an acetylating agent with or without catalyst. El Seoud et al. [160] successively acetylated cellulose from cotton linters, sisal, and SCB under homogeneous reaction conditions in DMAc/LiCl and the procedure (shown in Fig. 5.35) is as follows: (i) cellulose and LiCl were heated at 110 °C under reduced pressure; (ii) cellulose was dissolved in DMAc/LiCl by heating at 155 °C, followed by cooling to 40 °C; (iii) the solubilized polymer was acylated at 60 °C for 18 h. Attractive features of this procedure is the easy control and high reproducibility of the DS, the elimination of base catalyst, the negligible degradation of the natural polymer, and the recycling of high purity DMAc and acetic anhydride.

The homogeneous acetylation of cellulose was successfully performed in various ILs under mild conditions with the development of the cellulose dissolution in ILs in recent years. Wu et al. [172, 173] first reported the homogeneous acetylation of cellulose in ILs. They found that the homogeneous acetylation with acetic anhydride could be

carried out in AmimCl without any catalyst, and cellulose acetates with a wide range of DS (0.94–2.74) could be obtained under different conditions. Heinze et al. [122] found that it was very easy to synthesize cellulose acetate with high DS in good yield within a short time using different ILs such as [C<sub>4</sub>mim]Cl as reaction media and using acetyl chloride or acetic anhydride as acetylation reagent without any catalysts. The reaction of three free hydroxyl groups in cellulose at the C2, C3, and C6 positions all occur during homogeneous modification in ILs, and the order of reactivity is C6-OH > C3-OH > C2-OH [173] similar to that observed in acetylation in DMAc/LiCl system [160]. Cao et al. [174] prepared cornhusk cellulose acetates with DS ranging from 2.16 to 2.63 in AmimCl. The resultant cellulose acetates were readily dissolved in some organic solvents, such as acetone and DMSO. The distribution of the acetyl moiety among the three OH groups of the AGU revealed a preference at the C6 position. Barthel and Heinze [175] investigated different ILs as reaction media for the homogeneous acylation of cellulose. They found that cellulose acetates with DS in the range from 2.5 to 3.0 were accessible in [C<sub>4</sub>mim]Cl within 2 h at 80 °C in a complete homogeneous procedure. Ionic liquid analogues based on choline chloride (ChCl; HOCH<sub>2</sub>CH<sub>2</sub>N(Me)<sub>3</sub>Cl) and zinc chloride have been successfully used for a variety of reactions [128–130]. Abbott et al. [129] demonstrated the efficient acetylation of cellulose with acetic anhydride for 3 h at 90 °C using this Lewis acidic ionic liquid based on choline chloride and zinc chloride to achieve cellulose acetate with low levels of functionalization (<20% acetylation).

Because of its limited solubility, cellulose triacetate has not been used in a great number of commercial applications, whereas cellulose diacetates after deacetylation of triacetate are industrially more important. The acidic properties of the molten inorganic hydrates could be used for the cleavage of functional groups. Cellulose triacetate deacetylation can be



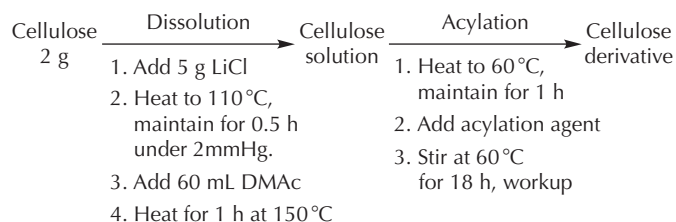


FIGURE 5.35 Procedure for cellulose acetylation in DMAc/LiCl [160].

carried out in molten  $\text{ZnCl}_2 \cdot 4\text{H}_2\text{O}$ . After a reaction time of 21 h, DS of 1.81 and PDS (C6) of 0.53 were obtained [176]. Furthermore, molten  $\text{ZnCl}_2 \cdot 4\text{H}_2\text{O}$  or  $\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$  can be applied as medium for deprotection of triphenylmethyl cellulose. A complete deprotection occurs in the comparatively short reaction time of 3–5 h [177].

### 5.5.2 Acylation with Other Linear Anhydrides or Chlorides

A number of reports exist in the literature pertaining to the preparation of esters of cellulose with the ultimate aim of significantly increased hydrophobic properties of the polymer and imparting suitable mechanical characteristics such as to render it more useful as engineering materials than the precursor polymer, which could be achieved by acylation with linear anhydrides or chloride. El Seoud et al. [160] successively acylated cellulose samples from cotton linters, sisal, and sugar cane bagasse to prepare cellulose esters including acetate, propionate, butyrate, and acetate/butyrate in DMAc/LiCl under homogeneous reaction conditions according to the procedure shown in Fig. 5.35. The DS of cellulose derivatives is listed in Table 5.6. The results showed that the efficiency of the esterification decreased with the increasing chain length of the anhydrides. As shown in Table 5.6, the reproducibility of DS is easily attainable in homogeneous acylation of cellulose in DMAc/LiCl, which is very difficult in heterogeneous derivatization. Barthel and Heinze [175] prepared cellulose laurates with DS from 0.34 to 1.54 with lauroyl chloride in ILs. They found that the acylation started homogeneously and continued heterogeneously in  $[\text{C}_4\text{mim}]\text{Cl}$ . The acylation efficiency of cellulose with lauroyl chloride decreased compared with acetylation.

### 5.5.3 Esterification with Cyclic Anhydrides

One of the key properties required for the cellulose materials intended to be used in hygienic products is their ability to absorb liquids. The absorbent hydrogels are lightly cross-linked hydrophilic polymers capable of absorbing large amounts of water as much as 10–1000 times their own weight, which have been widely used in various

applications such as disposal diapers, sanitary napkins, additives for soil in agriculture, and medicine for drug delivery systems. Most commercially available superabsorbent hydrogels are cross-linked sodium polyacrylates with extremely high molecular weights, but they cannot be biodegraded. Thus, their use may result in environmental pollution because their major applications are in disposable goods. Therefore, biodegradable superabsorbent hydrogels as a substitute for conventional synthetic polymers have been actively studied in recent years [178].

Carboxylic acids are known to improve water absorption of the macromolecular cellulose. Acylation of cellulose fibers with cyclic anhydrides results in cellulose derivatives with carboxylic acid group attached onto cellulose molecule, which has the potential as water absorbents for soil in agriculture, natural absorbents for the removal of heavy metal ions in wastewater treatment, medicine for drug delivery systems, thermoplastic materials, and superabsorbent hydrogels [178, 179]. It should be noted that modification with linear chain anhydrides produces an undesired by-product, namely the corresponding carboxylic acid. This acid must be removed from the reaction following modification. However, modification with cyclic anhydrides such as succinic anhydride does not yield a by-product. Liu et al. [180, 181] accomplished the succinoylation of SCB cellulose with SA using ILs as a reaction medium without any catalysts. The cellulose derivatives with DS ranging from 0.071 to 0.22 were obtained in AmimCl [181] and from 0.037 to 0.53 in  $[\text{C}_4\text{mim}]\text{Cl}$  [180]. These results indicated that chemical modification of cellulose with solid succinic anhydride in ILs only resulted in cellulose derivatives with relatively low DS. It was found that the crystallinity of cellulose was completely disrupted. Cellulose degradation during dissolution and derivatization was also reported [181–183]. Hadano et al. [179] modified four types of waste pulps with succinic anhydride (SA) and maleic anhydride (MA). The results showed that the reaction using SA proceeded more easily than the reaction using MA, and the DS values of Pulp-SA became higher than those of Pulp-MA. Because of the double bond of MA, MA is more stable than SA. Thus, it may be expected that pulp reacted more easily to SA than to MA. The chemical modification of cellulose with phthalic anhydride (PA) in  $[\text{C}_4\text{mim}]\text{Cl}$  and AmimCl was also achieved [182, 183], and phthalated cellulosic derivatives with DS ranging from

**TABLE 5.6** Acylation of Bagasse, Cotton, and Sisal Celluloses [160]

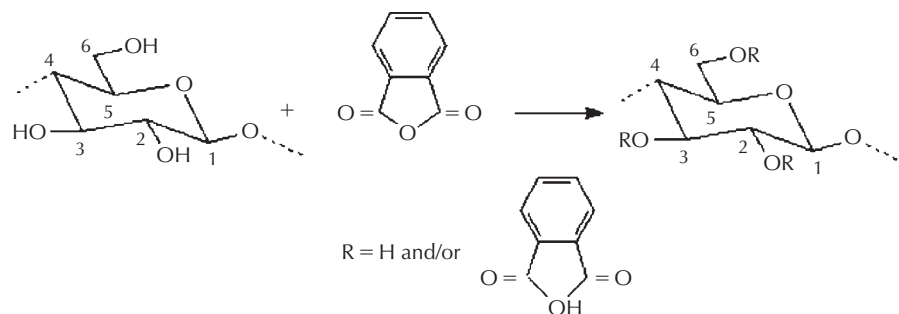
Cellulose	Targeted acyl derivative	Molar ratio of anhydride/AGU	Mean DS ( $\pm 0.1$ )
Bagasse	monoacetate	1.5	1.0
	diacetate	3.0	2.1
	triacetate	4.5	2.9
	monopropionate	2.0	1.1
	dipropionate	3.0	2.1
	monobutyrate	1.5	0.9
	dibutyrate	3.0	1.7
cotton	monoacetate	1.5	0.9
	diacetate	3.0	1.9
	triacetate	4.5	2.8
	monopropionate	1.5	0.9
	dipropionate	3.0	1.8
	monobutyrate	1.5	0.8
	dibutyrate	3.0	1.7
Sisal	monoacetate	1.5	1.0
	diacetate	3.0	2.0
	triacetate	4.5	2.8
	monopropionate	1.5	1.0
	dipropionate	3.0	2.0
	monobutyrate	1.5	0.9
	tributyrate	4.5	2.8

0.12 to 2.54 in  $[\text{C}_4\text{mim}]\text{Cl}$  and 0.10 to 0.73 in  $\text{AmimCl}$ , respectively, were prepared. The results indicated that phthalation of cellulose in  $[\text{C}_4\text{mim}]\text{Cl}$  resulted in the production of cellulosic monoester, as shown in Fig. 5.36. Solid-state CP/MAS  $^{13}\text{C}$ -NMR analysis indicated that the reaction of hydroxyl groups at C-6, C-2, and C-3 positions of cellulose has occurred (Fig. 5.37).

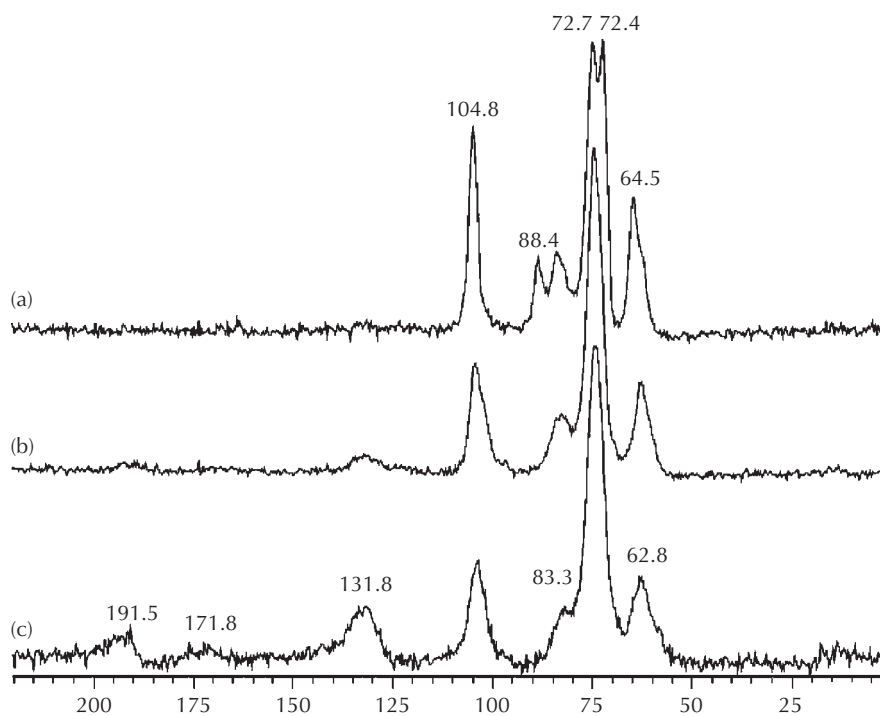
The homogeneous chemical modification of cellulose with succinic anhydride was investigated in a solvent containing  $[\text{C}_4\text{mim}]\text{Cl}$  and DMSO using *N*-bromosuccinimide (NBS) as a catalyst. Figure. 5.38 shows the reaction of SCB cellulose with SA using NBS as a catalyst in  $[\text{C}_4\text{mim}]\text{Cl}/\text{DMSO}$ . The results showed that the DS of cellulose succinates was in the range of 0.24–2.31, and it noticeably increased compared with the products without any catalysts, as shown in FTIR and CP/MAS  $^{13}\text{C}$ -NMR spectra in Figs 5.39 and 5.40, indicating that NBS was a novel efficient catalyst for cellulose succinylation in ILs. The possible mechanism of NBS in cellulose succinylation in ILs is shown in Fig. 5.41.

### 5.5.4 Carboxymethylation

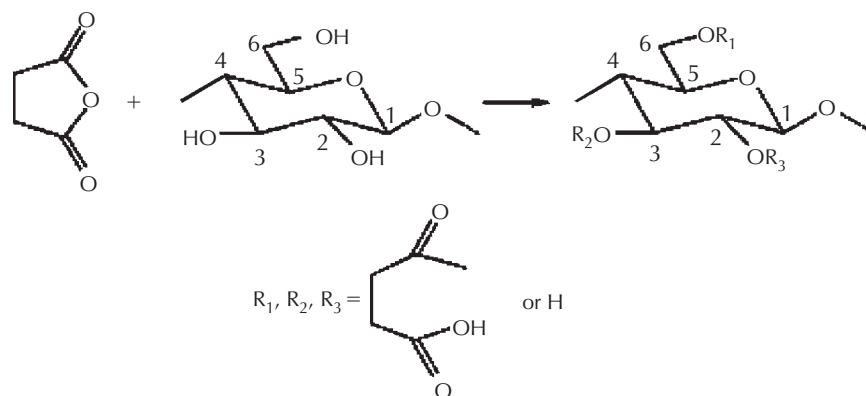
Carboxymethylation is one of the most versatile functionalization procedures as it provides access to bio-based materials with valuable properties such as filming, emulsification, suspension, water maintaining, and binding. [185]. Carboxymethylated polymers have been used for many applications, such as printing and dyeing, medicine, food, textures, toilet, oil drilling, electrical elements, and paper-making. Sodium carboxymethylcellulose (CMC), the product of cellulose carboxymethylation, is a linear, long-chain, water-soluble, anionic, man-modified cellulose derivative. Purified CMC is a white-colored to cream-colored, tasteless, odorless, free-flowing powder. In general, sodium CMC was produced by conversion of alkali cellulose swollen in aqueous NaOH and a surplus of organic solvent (e.g. isopropanol or ethanol) with monochloroacetic acid or its sodium salt [186]. In this case, hydroxyl groups in cellulose are usually replaced by carboxymethyl groups in the order of  $\text{C}2 > \text{C}6 > \text{C}3$  [187]. Adinugraha et al. [20] prepared CMC



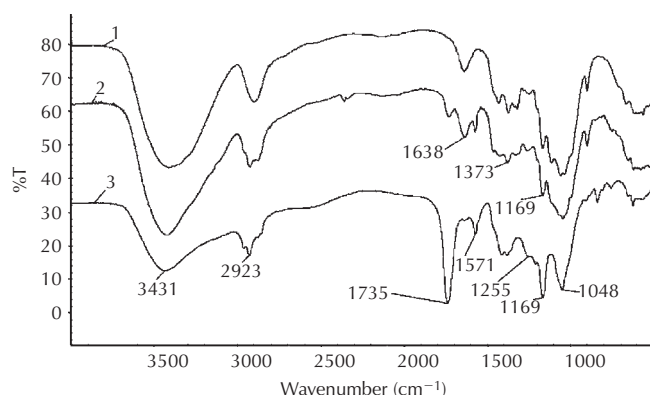
**FIGURE 5.36** Reaction of SCB cellulose with phthalic anhydride [182, 183].



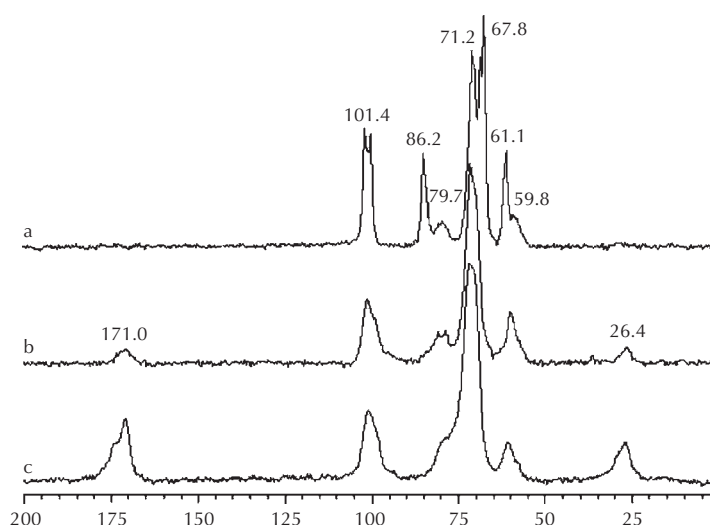
**FIGURE 5.37** Solid state CP/MAS  $^{13}\text{C}$  NMR spectra of unmodified cellulose (spectrum (a)) and phthalated cellulosic samples (spectrum (b), DS 0.21; spectrum (c), DS 2.54) [182].



**FIGURE 5.38** Reaction of SCB cellulose with SA using NBS as a catalyst [184].



**FIGURE 5.39** FTIR spectra of unmodified cellulose (spectrum 1) and succinylated cellulose without any catalyst (spectrum 2) and with NBS catalyst (spectrum 3) [184].



**FIGURE 5.40** Solid state CP/MAS  $^{13}\text{C}$  NMR spectra of unmodified cellulose (spectrum a) and succinylated cellulose without any catalysts (spectrum b) and with NBS as a catalyst (spectrum c) [184].

from cavendish banana pseudo stem cellulose according to the conventional procedure. The results showed that CMC with the highest DS, viscosity, purity, and crystallinity was obtained by the alkalization using 15% NaOH and etherification using 1.2 g (w/w)  $\text{ClCH}_2\text{COONa}$ .

The homogeneous carboxymethylation of cellulose in inorganic molten salts such as  $\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$  is possible using sodium monochloroacetate in the presence of NaOH [164, 188]. Fischer et al. [93] investigated carboxymethylation of cellulose in different inorganic molten salts. A representative overview of the carboxymethylation results is given in Fig. 5.42. CMC with DS values as high as 2 could be prepared within a short reaction time (4 h). A distribution of substituents on the level of the AGU in the order  $\text{C-6} > \text{C-2} \approx \text{C-3}$  was discovered, and the investigations showed that a complete substitution at position O-6 is possible.

Heinze et al. [186] investigated carboxymethylation of cellulose in different solvents including  $\text{Ni}(\text{tren})(\text{OH})_2$  [tren = tris(2-aminoethyl)amine],  $\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$ , and

NMMO. In case of  $\text{Ni}(\text{tren})(\text{OH})_2$ , a totally homogeneous carboxymethylation of cellulose with sodium monochloroacetate in the presence of an aqueous NaOH solution is possible. The results revealed the functionalization of the hydroxyl groups in the order  $\text{C-6} \geq \text{C-2} > \text{C-3}$ . The carboxymethylation of cellulose in  $\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$  was shown to be possible and yielded products of a statistic functionalization pattern as well. Carboxymethylation of cellulose in pure NMMO or NMMO/DMSO mixtures yielded CMC samples with a nonstatistical distribution of functional groups along with the polymer chain; this result is comparable to the earlier findings that etherification reactions start from a solution of cellulose in DMA/LiCl or from cellulose intermediates in DMSO.

Carboxymethylation of cellulose in IL  $[\text{C}_4\text{mim}]\text{Cl}$  was proposed by Heinze et al. [122]. However, carboxymethylated cellulose obtained according to this method had relatively low DS, and an increase in DS was not observed along with the increase in the dosage of carboxymethylating reagent. The reason for this unusual result is still unclear.

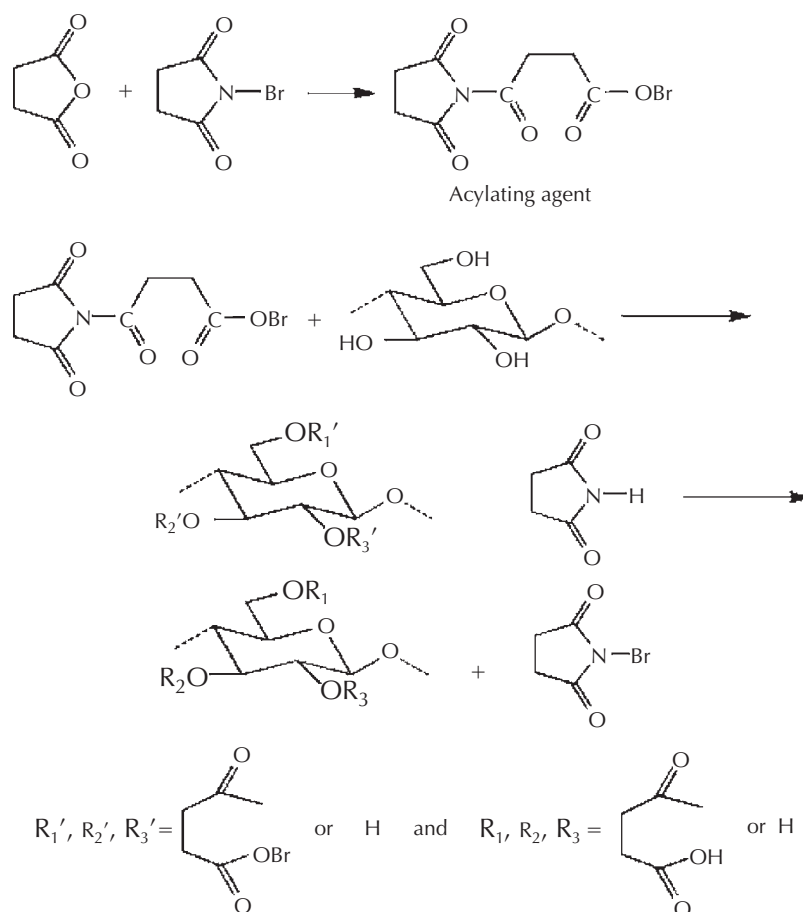


FIGURE 5.41 Mechanism of succinylation of cellulose using NBS as a catalyst [184].

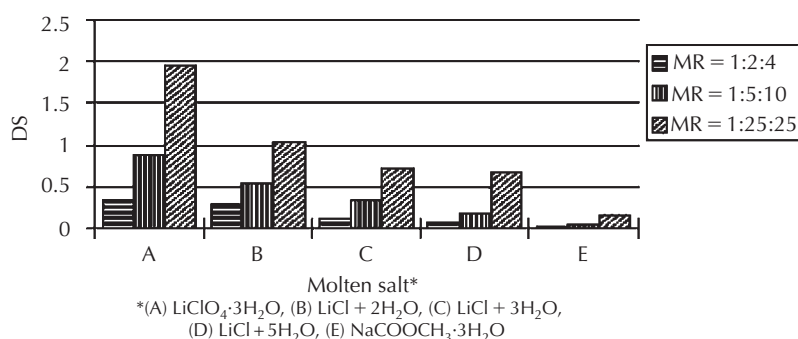


FIGURE 5.42 The degree of substitution (DS) of carboxymethylcellulose prepared in molten salts plotted as function of salt hydrate species, water content, and molar ratio MR (anhydroglucose unit:sodium monochloroacetate: NaOH) [93].

### 5.5.5 Other Chemical Modification

Except for acylation with linear anhydride and linear chloride, esterification with cyclic anhydrides, and carboxymethylation, there are several other chemical modifications of cellulose reported in recent years, including tritylation, tosylation, quaternization, carbanilation, and so on. These modifications provide the novel utilization of cellulose, the

protection of hydroxy groups, and the structural elucidation of cellulose products.

Erdmenger et al. [123] performed the tritylation of cellulose in  $[\text{C}_4\text{mim}]\text{Cl}$  using pyridine as base, as shown in Fig. 5.43. A DS of around 1 was obtained after the reaction time of 3 h using a six-fold excess of trityl chloride. Granstrom et al. [189] performed a series of cellulose modification including esterification with *pyro*-pheophorbide



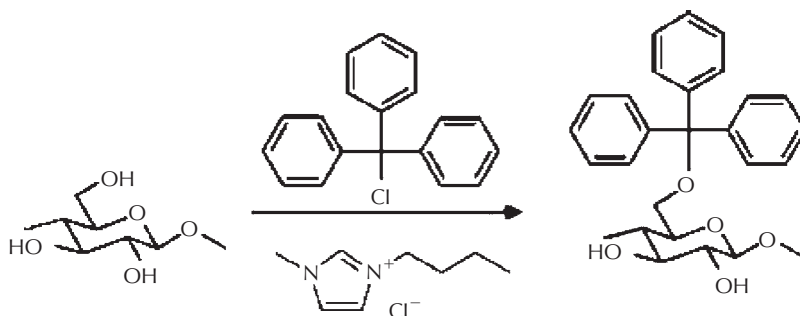


FIGURE 5.43 Tritylation of cellulose in [C<sub>4</sub>mim]Cl using pyridine as base [123].

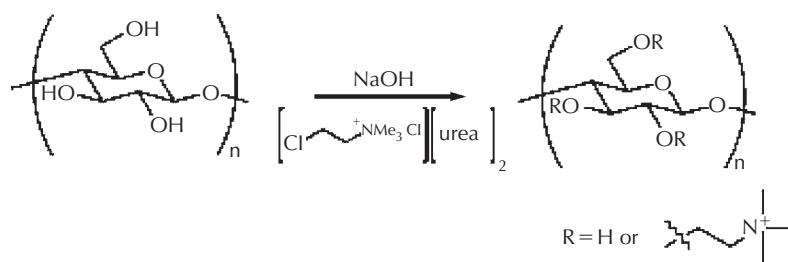


FIGURE 5.44 Cellulose quaternization in ClCHCl/urea at 90 °C for 15 h [131].

activated with *N,N'*-carbonyldiimidazole (CDI), esterification with stearic acid activated with 1-ethyl-3-(3'-dimethylamino-propyl) carbodiimide hydrochloride (EDCI), triacetylation with acetic anhydride catalyzed with pyridine, and tosylation with tosyl chloride catalyzed with pyridine using AmimCl as solvents and etherification of tosylcellulose with 11-Bromoundecanol in DMF. The cellulose derivatives with DS of 0.07, 0.16, 2.99, 0.84, and 0.73, respectively, were obtained. Abbott et al. [131] found the efficient cationic functionalization of cellulose using an ionic liquid analogue, based on a eutectic mixture of a choline chloride derivative and urea, as both solvent and reagent. The results showed that all the available hydroxyl groups on cellulose had been quaternized at 90 °C for 15 h. The derivatization reaction of cellulose with cationic functionalities is shown in Fig. 5.44.

Using the solvent DMSO/TBAF as reaction medium, the homogenous modification of cellulose, including acylation with acetic anhydride, vinyl acetate, vinyl butyrate, vinyl laurate, and benzoate, tosylation with tosylchloride in the presence of triethylamine, and carboxymethylation with sodium monochloroacetate, was comparatively studied [136]. Cellulose esters obtained from modification with acetic anhydride, vinyl acetate, vinyl butyrate, vinyl laurate, and vinyl benzoate had the DS of 0.83, 1.04, 0.86, 1.47, and 0.95, respectively. Barthel and Heinze [175] investigated the homogeneous carbanilation of cellulose in different ILs. The synthesis of cellulose carbanilates succeeded in the ionic liquid [C<sub>4</sub>mim]Cl without any catalyst. Cellulose carbanilate with DS in the range of 0.26 to 3.0 could be obtained.

## 5.6 CONCLUDING REMARKS

At present, utilization of cellulose has received much attention for the production of biofuels, chemicals, and composites as alternatives of the fossil fuel-based products. Straws represent a large amount of natural, renewable, inexpensive, biodegradable, and readily available resources of cellulose. Isolation of cellulose with high yield and purity is a long-standing goal in cellulose development because of the complexity of cell wall structure of straws. Furthermore, straw cellulose provides the important potential utilization for the regenerated cellulose, biofuels such as bioethanol, and a series of cellulose derivatives. Homogeneous derivatization of cellulose in green media is the trend in straw cellulose utilization.

## REFERENCES

- [1] D. Klemm, B. Heublein, H.P. Fink, A. Bohn, Cellulose: fascinating biopolymer and sustainable raw material, *Angew. Chem. Int. Ed.* 44 (2005) 3358–3393.
- [2] Y. Cao, J. Wu, J. Zhang, H.Q. Li, Y. Zhang, J.S. He, Room temperature ionic liquids (RTILs): a new and versatile platform for cellulose processing and derivatization, *Chem. Eng. J.* 147 (2009) 13–21.
- [3] N. Reddy, Y. Yang, Biofibers from agricultural byproducts for industrial applications, *Trends Biotechnol.* 23 (2005) 22–27.
- [4] M. Knauf, M. Moniruzzaman, Lignocellulosic biomass processing: a perspective, *Int. Sugar J.* 106 (2004) 147–150.
- [5] R.C. Sun, J. Tomkinson, Essential guides for isolation/purification of polysaccharides, *Encyclopedia Sep. Sci.* 6 (2000) 4568–4574.

- [6] S.J. Hanley, J.F. Revol, L. Godbout, D.G. Gray, Atomic force microscopy and transmission electron microscopy of cellulose from *Micrasterias denticulata*; evidence for a chiral helical microfibril twist, *Cellulose* 4 (1997) 209–220.
- [7] H. Yu, R.G. Liu, D.W. Shen, Y. Jiang, Y. Huang, Study on morphology and orientation of cellulose in the vascular bundle of wheat straw, *Polymer* 46 (2005) 5689–5694.
- [8] J.C. Thimm, D.J. Burritt, W.A. Ducker, L.D. Melton, Celery (*Apium graveolens* L.) parenchyma cell walls examined by atomic force microscopy: effect of dehydration on cellulose microfibrils, *Planta* 212 (2000) 25–32.
- [9] N. Reddy, Y.Q. Yang, Preparation and characterization of long natural cellulose fibers from wheat straw, *J. Agric. Food Chem.* 55 (2007) 8570–8575.
- [10] C.F. Liu, F. Xu, J.X. Sun, J.L. Ren, S. Curling, R.C. Sun, P. Fowler, M.S. Baird, Physicochemical characterization of cellulose from perennial ryegrass leaves (*Lolium perenne*), *Carbohydr. Res.* 341 (2006) 2677–2687.
- [11] O. Brendel, P.P.M. Iannetta, D. Stewart, A rapid and simple method to isolate pure alpha cellulose, *Phytochem. Anal.* 11 (2000) 7–10.
- [12] R.C. Sun, S. Hughes, Fractional extraction and physico chemical characterization of hemicelluloses and cellulose from sugar beet pulp, *Carbohydr. Polym.* 36 (1998) 293–299.
- [13] N.J. Loader, I. Robertson, A.C. Barker, V.R. Switsur, J.S. Waterhouse, An improved technique for the batch processing of small wholewood samples to alpha cellulose, *Chem. Geol.* 136 (1997) 313–317.
- [14] R.C. Sun, X.F. Sun, J. Tomkinson, Hemicelluloses and their derivatives, in: P. Gatenholm, M. Tenhunen (Eds.), *Hemicelluloses: Science and Technology*, 2004, pp. 2–22.
- [15] A.J. Buchala, C.G. Fraser, K.C.B. Wilkie, Extraction of hemicellulose from oat tissues during the process of delignification, *Phytochemistry* 11 (1972) 1249–1254.
- [16] A. Hoijs, M. Grondahl, K. Tommeraa, P. Gatenholm, Isolation and characterization of physicochemical and material properties of arabinoxylans from barley husks, *Carbohydr. Polym.* 61 (2005) 266–275.
- [17] R.C. Sun, J.M. Lawther, W.B. Banks, Isolation and characterization of hemicellulose B and cellulose from pressure refined wheat straw, *Ind. Crops Prod.* 7 (1998) 121–128.
- [18] B. Xiao, X.F. Sun, R.C. Sun, Chemical, structural, and thermal characterizations of alkali soluble lignins and hemicelluloses, and cellulose from maize stems, rye straw, and rice straw, *Polym. Degrad. Stab.* 74 (2001) 307–319.
- [19] R.C. Sun, J.M. Fang, P. Rowlands, J. Bolton, Physicochemical and thermal characterization of wheat straw hemicelluloses and cellulose, *J. Agric. Food Chem.* 46 (1998) 2804–2809.
- [20] M.P. Adinugraha, D.W. Marseno, Haryadi, Synthesis and characterization of sodium carboxymethylcellulose from cavendish banana pseudo stem (*Musa cavendishii* LAMBERT), *Carbohydr. Polym.* 62 (2005) 164–169.
- [21] A. Alemdar, M. Sain, Isolation and characterization of nanofibers from agricultural residues Wheat straw and soy hulls, *Bioresour. Technol.* 99 (2008) 1664–1671.
- [22] T.J. Mason, A. Newman, J.P. Lorimer, J. Lindley, K. Hutt, Ultrasonically assisted catalytic decomposition of aqueous sodium hypochlorite, *Ultrason. Sonochem.* 3 (1996) 53–55.
- [23] C.F. Liu, J.L. Ren, F. Xu, J.J. Liu, J.X. Sun, R.C. Sun, Isolation and characterization of cellulose obtained from ultrasonic irradiated sugarcane bagasse, *J. Agric. Food Chem.* 54 (2006) 5742–5748.
- [24] T. Josefsson, H. Lennholm, G. Gellerstedt, Changes in cellulose supramolecular structure and molecular weight distribution during steam explosion of aspen wood, *Cellulose* 8 (2001) 289–296.
- [25] T. Josefsson, H. Lennholm, G. Gellerstedt, Steam explosion of aspen wood. Characterisation of reaction products, *Holzforchung* 56 (2002) 289–297.
- [26] X.F. Sun, F. Xu, R.C. Sun, P. Fowler, M.S. Baird, Characteristics of degraded cellulose obtained from steam exploded wheat straw, *Carbohydr. Res.* 340 (2005) 97–106.
- [27] X.F. Sun, R.C. Sun, J. Tomkinson, M.S. Baird, Isolation and characterization of lignins, hemicelluloses, and celluloses from wheat straw by alkaline peroxide treatment, *Cellul. Chem. Technol.* 37 (2003) 283–304.
- [28] J.M. Fang, R.C. Sun, J. Tomkinson, Isolation and characterization of hemicelluloses and cellulose from rye straw by alkaline peroxide extraction, *Cellulose* 7 (2000) 87–107.
- [29] X.F. Sun, R.C. Sun, P. Fowler, M.S. Baird, Isolation and characterisation of cellulose obtained by a two stage treatment with organosolv and cyanamide activated hydrogen peroxide from wheat straw, *Carbohydr. Polym.* 55 (2004) 379–391.
- [30] N. Cordeiro, C.P. Neto, J. Rocha, M.N. Belgacem, A. Gandini, The organosolv fractionation of cork components, *Holzforchung* 56 (2002) 135–142.
- [31] X.J. Pan, Y. Sano, Atmospheric acetic acid pulping of rice straw IV: physico chemical characterization of acetic acid lignins from rice straw and woods. part 2. Chemical structures, *Holzforchung* 53 (1999) 590–596.
- [32] X.J. Pan, Y. Sano, Atmospheric acetic acid pulping of rice straw IV: physico chemical characterization of acetic acid lignins from rice straw and woods. Part 1. Physical characteristics, *Holzforchung* 53 (1999) 511–518.
- [33] X.J. Pan, Y. Sano, Acetic acid pulping of wheat straw under atmospheric pressure, *J. Wood Sci.* 45 (1999) 319–325.
- [34] X.J. Pan, Y. Sano, T. Ito, Atmospheric acetic acid pulping of rice straw II: behavior of ash and silica in rice straw during atmospheric acetic acid pulping and bleaching, *Holzforchung* 53 (1999) 49–55.
- [35] H.Q. Lam, Y.L. Bigot, M. Delmas, E.G. Avignon, Formic acid pulping of rice straw, *Ind. Crops Prod.* 14 (2001) 65–71.
- [36] R. Lehtinen, B. Saake, H.H. Nimz, Impact of pulping conditions on FORMACELL aspen lignin: investigation of methoxyl and ester groups, carbohydrates, molar mass and glass transition temperatures, *Holzforchung* 56 (2002) 498–506.
- [37] S. Abad, B. Saake, J. Puls, J.C. Parajo, Totally chlorine free bleaching of Eucalyptus globulus dissolving Pulps delignified with peroxyformic acid and formic acid, *Holzforchung* 56 (2002) 60–66.
- [38] E.W. Crampton, L.A. Maynard, The relation of cellulose and lignin content to the nutritive value of animal feeds, *J. Nutr.* 15 (1938) 383–395.
- [39] X.F. Sun, R.C. Sun, J. Tomkinson, M.S. Baird, Degradation of wheat straw lignin and hemicellulosic polymers by a totally chlorine free method, *Polym. Degrad. Stab.* 83 (2004) 47–57.
- [40] F. Xu, R.C. Sun, Q. Lu, Characteristics of cellulose isolated by a totally chlorine free method from Caragana korshinskii, *J. Appl. Polym. Sci.* 101 (2006) 3251–3263.
- [41] F. Xu, J.X. Sun, R.C. Sun, Characteristics of cellulose isolated by a totally chlorine free method from fast growing poplar wood, oil palm frond fibre and cereal straws, *Cellul. Chem. Technol.* 39 (2005) 3–23.
- [42] J.X. Sun, X.F. Sun, H. Zhao, R.C. Sun, Isolation and characterization of cellulose from sugarcane bagasse, *Polym. Degrad. Stab.* 84 (2004) 331–339.

- [43] X.F. Sun, R.C. Sun, Y.Q. Su, J.X. Sun, Comparative study of crude and purified cellulose from wheat straw, *J. Agric. Food Chem.* 52 (2004) 839–847.
- [44] J.X. Sun, F. Xu, X.F. Sun, B. Xiao, R.C. Sun, Physico chemical and thermal characterization of cellulose from barley straw, *Polym. Degrad. Stab.* 88 (2005) 521–531.
- [45] R.C. Sun, J. Tomkinson, Separation and characterization of cellulose from wheat straw, *Sep. Sci. Technol.* 39 (2004) 391–411.
- [46] J.X. Sun, F. Xu, Z.C. Geng, X.F. Sun, R.C. Sun, Comparative study of cellulose isolated by totally chlorine free method from wood and cereal straw, *J. Appl. Polym. Sci.* 97 (2005) 322–335.
- [47] J.S. Zhao, C.G. Fu, Z.Y. Yang, Integrated process for isolation and complete utilization of rice straw components through sequential treatment, *Chem. Eng. Commun.* 195 (2008) 1176–1183.
- [48] D. Bhattacharya, L.T. Germinario, W.T. Winter, Isolation, preparation and characterization of cellulose microfibrils obtained from bagasse, *Carbohydr. Polym.* 73 (2008) 371–377.
- [49] M. Kikuchi, K. Ogawa, T. Yamazaki, S. Kajiwar, K. Shishido, Use of molecular genetically bred *Coprinus cinereus* strains for an efficient isolation of cellulose from rice straw, *Biosci. Biotechnol. Biochem.* 66 (2002) 199–201.
- [50] S. Ates, C. Atik, Y.H. Ni, E. Gumuskaya, Comparison of different chemical pulps from wheat straw and bleaching with xylanase pre treated ECF Method, *Turk. J. Agric. For.* 32 (2008) 561–570.
- [51] J.F. Kadla, R.D. Gilbert, Cellulose structure: a review, *Cellul. Chem. Technol.* 34 (2000) 197–216.
- [52] H.A. Krassig, *Cellulose Structure, Accessibility and Reactivity*, Gordon Breach Science Publishers, Langhorne, PA, 1993.
- [53] A.W. Zykwinska, M.C.J. Ralet, C.D. Garnier, J.F.J. Thibault, Evidence for in vitro binding of pectin side chains to cellulose, *Plant Physiol.* 139 (2005) 397–407.
- [54] M. Wada, L. Heux, J. Sugiyama, Polymorphism of cellulose I family: reinvestigation of cellulose IV, *Biomacromolecules* 5 (2004) 1385–1391.
- [55] X.H. Qian, S.Y. Ding, M.R. Nimlos, D.K. Johnson, M.E. Himmel, Atomic and electronic structures of molecular crystalline cellulose I beta: a first principles investigation, *Macromolecules* 38 (2005) 10580–10589.
- [56] J. Hayashi, H. Kon, M. Takai, M. Hatano, T. Nozawa, The structure of cellulose, *ACS Symp. Ser.* 340 (1987) 134–136.
- [57] M. El Sakhawy, M.L. Hassan, Physical and mechanical properties of microcrystalline cellulose prepared from agricultural residues, *Carbohydr. Polym.* 67 (2007) 1–10.
- [58] P.T. Larsson, P.O. Westlund, Line shapes in CP/MAS C 13 NMR spectra of cellulose I, *Spectrochim. Acta A* 62 (2005) 539–546.
- [59] A. Thygesen, J. Oddershede, H. Lilholt, A.B. Thomsen, K. Stahl, On the determination of crystallinity and cellulose content in plant fibres, *Cellulose* 12 (2005) 563–576.
- [60] R.G. Liu, H. Yu, Y. Huang, Structure and morphology of cellulose in wheat straw, *Cellulose* 12 (2005) 25–34.
- [61] T. Imai, J. Sugiyama, Nanodomains of I alpha and I beta cellulose in algal microfibrils, *Macromolecules* 31 (1998) 6275–6279.
- [62] A. Sturcova, I. His, D.C. Apperley, J. Sugiyama, M.C. Jarvis, Structural details of crystalline cellulose from higher plants, *Biomacromolecules* 5 (2004) 1333–1339.
- [63] B. Focher, M.T. Palma, M. Canetti, G. Torri, C. Cosentino, G. Gastaldi, Structural differences between non wood plant celluloses: evidence from solid state NMR, vibrational spectroscopy and X ray diffractometry, *Ind. Crops. Prod.* 13 (2001) 193–208.
- [64] J. Sugiyama, R. Vuong, H. Chanzy, Electron diffraction study on the two crystalline phases occurring in native cellulose from an algal cell wall, *Macromolecules* 24 (1991) 4168–4175.
- [65] Y. Nishiyama, J. Sugiyama, H. Chanzy, P. Langan, Crystal structure and hydrogen bonding system in cellulose I(alpha), from synchrotron X ray and neutron fiber diffraction, *J. Am. Chem. Soc.* 125 (2003) 14300–14306.
- [66] R. Hori, M. Wada, The thermal expansion of wood cellulose crystals, *Cellulose* 12 (2005) 479–484.
- [67] J. Sugiyama, J. Persson, H. Chanzy, Combined infrared and electron diffraction study of the polymorphism of native celluloses, *Macromolecules* 24 (1991) 2461–2466.
- [68] H. Yamamoto, F. Horii, CP/MAS carbon 13 NMR analysis of the crystal transformation induced for Valonia cellulose by annealing at high temperatures, *Macromolecules* 26 (1993) 1313–1317.
- [69] H. Yamamoto, F. Horii, H. Odani, Structural changes of native cellulose crystals induced by annealing in aqueous alkaline and acidic solutions at high temperatures, *Macromolecules* 22 (1989) 4130–4132.
- [70] R.H. Atalla, D.L. VanderHart, Native cellulose: a composite of two distinct crystalline forms, *Science* 223 (1984) 283–285.
- [71] E.L. Hult, T. Iversen, J. Sugiyama, Characterization of the supermolecular structure of cellulose in wood pulp fibres, *Cellulose* 10 (2003) 103–110.
- [72] M. Wada, T. Okano, J. Sugiyama, F. Horii, Characterization of tension and normally lignified wood cellulose in *Populus maximowiczii*, *Cellulose* 2 (1995) 223–233.
- [73] W. Li, L.F. Yan, J.L. Yang, AFM study of crystalline cellulose in the cell walls of straw, *Polym. Int.* 55 (2006) 87–92.
- [74] V.L. Finkenstadt, R.P. Millane, Crystal structure of Valonia cellulose I beta, *Macromolecules* 31 (1998) 7776–7783.
- [75] E. Dinand, M. Vignon, H. Chanzy, L. Heux, Mercerization of primary wall cellulose and its implication for the conversion of cellulose I > cellulose II, *Cellulose* 9 (2002) 7–18.
- [76] M.A. Moharram, O.M. Mahmoud, X ray diffraction methods in the study of the effect of microwave heating on the transformation of cellulose I into cellulose II during mercerization, *J. Appl. Polym. Sci.* 105 (2007) 2978–2983.
- [77] S. Raymond, A. Kvick, H. Chanzy, The structure of cellulose II: a revisit, *Macromolecules* 28 (1995) 8422–8425.
- [78] P. Langan, Y. Nishiyama, H. Chanzy, A revised structure and hydrogen bonding system in cellulose II from a neutron fiber diffraction analysis, *J. Am. Chem. Soc.* 121 (1999) 9940–9946.
- [79] A. Isogai, M. Usuda, T. Kato, T. Uryu, R.H. Atalla, Solid state CP/MAS carbon 13 NMR study of cellulose polymorphs, *Macromolecules* 22 (1989) 3168–3172.
- [80] R. Zuluaga, J.L. Putaux, J. Cruz, J. Velez, I. Mondragon, P. Ganan, Cellulose microfibrils from banana rachis: effect of alkaline treatments on structural and morphological features, *Carbohydr. Polym.* 76 (2009) 51–59.
- [81] R. Zuluaga, J.L. Putaux, A. Restrepo, I. Mondragon, P. Ganan, Cellulose microfibrils from banana farming residues: isolation and characterization, *Cellulose* 14 (2007) 585–592.
- [82] Y. Nishio, R.J. Manley, Cellulose/poly(vinyl alcohol) blends: an estimation of thermodynamic polymer polymer interaction by melting point depression analysis, *Macromolecules* 22 (1989) 2547–2549.
- [83] Y. Nishio, R.J. Manley, Cellulose poly(vinyl alcohol) blends prepared from solutions in N,N dimethylacetamide lithium chloride, *Macromolecules* 21 (1988) 1270–1277.

- [84] Y. Nishio, S.K. Roy, R.S.J. Manley, Blends of cellulose with polyacrylonitrile prepared from N,N dimethylacetamide lithium chloride solutions, *Polymer* 28 (1987) 1385–1390.
- [85] K. Hattori, E. Abe, T. Yoshida, J.A. Cuculo, New solvents for cellulose. II. Ethylenediamine/thiocyanate salt system, *Polym. J.* 36 (2004) 123–130.
- [86] H. Lennholm, T. Iversen, The effects of laboratory beating on cellulose structure, *Nord. Pulp Pap. Res. J.* 10 (1995) 104–108.
- [87] M.A. Moharram, O.M. Mahmoud, FTIR spectroscopic study of the effect of microwave heating on the transformation of cellulose I into cellulose II during mercerization, *J. Appl. Polym. Sci.* 107 (2008) 30–36.
- [88] A. Potthast, T. Rosenau, P. Kosma, Analysis of oxidized functionalities in cellulose, *Adv. Polym. Sci.* 205 (2006) 304–352.
- [89] L.Y. Liu, H.Z. Chen, Enzymatic hydrolysis of cellulose materials treated with ionic liquid [BMIM]Cl, *Chin. Sci. Bull.* 51 (2006) 2432–2436.
- [90] H. Zhang, J. Wu, J. Zhang, J.S. He, 1-Allyl-3-methylimidazolium chloride room temperature ionic liquid: a new and powerful nonderivatizing solvent for cellulose, *Macromolecules* 38 (2005) 8272–8277.
- [91] S.L. Maunu, NMR studies of wood and wood products, *Prog. Nucl. Magn. Reson. Spectrosc.* 40 (2002) 151–174.
- [92] F.C. Lu, J. Ralph, Non degradative dissolution and acetylation of ball milled plant cell walls: high resolution solution state NMR, *Plant J.* 35 (2003) 535–544.
- [93] S. Fischer, H. Leipner, K. Thummler, E. Brendler, J. Peters, Inorganic molten salts as solvents for cellulose, *Cellulose* 10 (2003) 227–236.
- [94] T. Rosenau, A. Hofinger, A. Potthast, P. Kosma, On the conformation of the cellulose solvent N-methylmorpholine N-oxide (NMMO) in solution, *Polymer* 44 (2003) 6153–6158.
- [95] J. Cai, L. Zhang, Rapid dissolution of cellulose in LiOH/Urea and NaOH/Urea aqueous solutions, *Macromol. Biosci.* 5 (2005) 539–548.
- [96] R.P. Swatloski, S.K. Spear, J.D. Holbrey, R.D. Rogers, Dissolution of cellulose with ionic liquids, *J. Am. Chem. Soc.* 124 (2002) 4974–4975.
- [97] S. Fischer, W. Voigt, K. Fischer, The behaviour of cellulose in hydrated melts of the composition LiX center dot nH<sub>2</sub>O (X = I, NO<sub>3</sub>, CH<sub>3</sub>COO, ClO<sub>4</sub>), *Cellulose* 6 (1999) 213–219.
- [98] K. Letters, Viskosimetrische Untersuchungen über die Reaktion von cellulose mit konzentrierten chlorzinklösungen, *Kolloidzeitschrift* 58 (1932) 229–235.
- [99] S. Kuga, The porous structure of cellulose gel regenerated from calcium thiocyanate solution, *J. Colloid Interface Sci.* 77 (1980) 413–417.
- [100] Z. Lewandowski, Some aspects of fibre formation from cellulose solutions in N-methylmorpholine N-oxide (NMMO), *Fibres Text. East. Eur.* 8 (2000) 24–28.
- [101] T. Rosenau, A. Potthast, I. Adorjan, A. Hofinger, H. Sixta, H. Firgo, P. Kosma, Cellulose solutions in N-methylmorpholine N-oxide (NMMO) degradation processes and stabilizers, *Cellulose* 9 (2002) 283–291.
- [102] H.P. Fink, P. Weigel, H.J. Purz, J. Ganster, Structure formation of regenerated cellulose materials from NMMO solutions, *Prog. Polym. Sci.* 26 (2001) 1473–1524.
- [103] C.H. Kuo, C.K. Lee, Enhanced enzymatic hydrolysis of sugarcane bagasse by N-methylmorpholine N-oxide pretreatment, *Bioresour. Technol.* 100 (2009) 866–871.
- [104] J. Cai, L.N. Zhang, J.P. Zhou, H.S. Qi, H. Chen, T. Kondo, X.M. Chen, B. Chu, Multifilament fibers based on dissolution of cellulose in NaOH/urea aqueous solution: structure and properties, *Adv. Mater.* 19 (2007) 821–825.
- [105] L. Zhang, J. Cai, J. Zhou, Solvent Composite and Preparation Method and Use Thereof, UNIV WUHAN (UYWU Non standard).
- [106] J. Cai, L. Zhang, Unique gelation behavior of cellulose in NaOH/Urea aqueous solution, *Biomacromolecules* 7 (2006) 183–189.
- [107] J. Cai, Y.T. Liu, L.N. Zhang, Dilute solution properties of cellulose in LiOH/urea aqueous system, *J. Polym. Sci. Polym. Phys.* 44 (2006) 3093–3101.
- [108] C. Graenacher, Cellulose Solution, U.S. Patent, 1943176, 1934.
- [109] E. Husemann, E. Siefert, N-Ethylpyridinium chloride as solvent and reaction medium for cellulose, *Macromol. Chem.* 128 (1969) 288–291.
- [110] B. Jastorff, R. Stormann, J. Ranke, K. Molter, F. Stock, B. Oberheitmann, W. Hoffmann, J. Hoffmann, M. Nuchter, B. Ondruschka, J. Filser, How hazardous are ionic liquids? Structure activity relationships and biological testing as important elements for sustainability evaluation, *Green Chem.* 2003 (2003) 136–142.
- [111] D.J. Gorman Lewis, J.B. Fein, Experimental study of the adsorption of an ionic liquid onto bacterial and mineral surfaces, *Environ. Sci. Technol.* 38 (2004) 2491–2495.
- [112] Q. Ren, J. Wu, J. Zhang, J.S. He, M.L. Guo, Synthesis of 1-allyl-3-methylimidazolium based room temperature ionic liquid and preliminary study of its dissolving cellulose, *Acta Polym. Sin.* (2003) 448–451.
- [113] T. Mizumo, E. Marwanta, N. Matsumi, H. Ohno, Allylimidazolium halides as novel room temperature ionic liquids, *Chem. Lett.* 33 (2004) 1360–1361.
- [114] H.M. Luo, Y.Q. Li, C.R. Zhou, Study on the dissolubility of the cellulose in the functionalized ionic liquid, *Polym. Mater. Sci. Eng.* 21 (2005) 233–235.
- [115] J. Zhang, Q. Ren, J. He, Cellulose Solution and Its Preparing Method, China Patent, ZL02147004.9, 2002.
- [116] F. Hermanutz, F. Gaehr, E. Uerdingen, F. Meister, B. Kosan, New developments in dissolving and processing of cellulose in ionic liquids, *Macromol. Symp.* 262 (2007) 23–27.
- [117] B. Kosan, C. Michels, F. Meister, Dissolution and forming of cellulose with ionic liquids, *Cellulose* 15 (2008) 59–66.
- [118] S.T. Handy, Greener solvents: room temperature ionic liquids from biorenewable sources, *Chem. Eur. J.* 9 (2003) 2938–2944.
- [119] Y. Fukaya, A. Sugimoto, H. Ohno, Superior solubility of polysaccharides in low viscosity, polar, and halogen free 1,3-dialkylimidazolium formates, *Biomacromolecules* 7 (2006) 3295–3297.
- [120] Y. Fukaya, K. Hayashi, M. Wada, H. Ohno, Cellulose dissolution with polar ionic liquids under mild conditions: required factors for anions, *Green Chem.* 10 (2008) 44–46.
- [121] E. Kuhlmann, S. Himmler, H. Giebelhaus, P. Wasserscheid, Imidazolium dialkylphosphates—a class of versatile, halogen free and hydrolytically stable ionic liquids, *Green Chem.* 9 (2007) 233–242.
- [122] T. Heinze, K. Schwikal, S. Barthel, Ionic liquids as reaction medium in cellulose functionalization, *Macromol. Biosci.* 5 (2005) 520–525.
- [123] T. Erdmenger, C. Haensch, R. Hoogenboom, U.S. Schubert, Homogeneous tritylation of cellulose in 1-butyl-3-methylimidazolium chloride, *Macromol. Biosci.* 7 (2007) 440–445.
- [124] J.P. Mikkola, A. Kirilin, J.C. Tuuf, A. Pranovich, B. Holmbom, L.M. Kustov, D.Y. Murzin, T. Salmi, Ultrasound enhancement of cellulose processing in ionic liquids: from dissolution towards functionalization, *Green Chem.* 9 (2007) 1229–1237.



- [125] B. Derecskei, A. Derecskei Kovacs, Molecular dynamic studies of the compatibility of some cellulose derivatives with selected ionic liquids, *Mol. Simul.* 32 (2006) 109–115.
- [126] T.G.A. Youngs, C. Hardacre, J.D. Holbrey, Glucose solvation by the ionic liquid 1,3 dimethylimidazolium chloride: a simulation study, *J. Phys. Chem. B* 111 (2007) 13765–13774.
- [127] J.S. Moulthrop, R.P. Swatloski, G. Moyna, R.D. Rogers, High resolution C 13 NMR studies of cellulose and cellulose oligomers in ionic liquid solutions, *Chem. Commun.* (2005) 1557–1559.
- [128] A.P. Abbott, G. Capper, D.L. Davies, R.H. Rasheed, V. Tambyrajah, Quaternary ammonium zinc or tin containing ionic liquids: water insensitive, recyclable catalysts for Diels Alder reactions, *Green Chem.* 4 (2002) 24–26.
- [129] A.P. Abbott, T.J. Bell, S. Handa, B. Stoddart, *O* acetylation of cellulose and monosaccharides using a zinc based ionic liquid, *Green Chem.* 7 (2005) 705–707.
- [130] R.C. Morales, V. Tambyrajah, P.R. Jenkins, D.L. Davies, A.P. Abbott, The regioselective Fischer indole reaction in choline chloride center dot 2ZnCl(2) with product isolation by direct sublimation from the ionic liquid, *Chem. Commun.* (2004) 158–159.
- [131] A.P. Abbott, T.J. Bell, S. Handa, B. Stoddart, Cationic functionalisation of cellulose using a choline based ionic liquid analogue, *Green Chem.* 8 (2006) 784–786.
- [132] A.P. Abbott, G. Capper, D.L. Davies, R.K. Rasheed, V. Tambyrajah, Novel solvent properties of choline chloride/urea mixtures, *Chem. Commun.* (2003) 70–71.
- [133] J. Araki, T. Kataoka, N. Katsuyama, A. Teramoto, K. Ito, K. Abe, A preliminary study for fiber spinning of mixed solutions of polyrotaxane and cellulose in a dimethylacetamide/lithium chloride (DMAc/LiCl) solvent system, *Polymer* 47 (2006) 8241–8246.
- [134] B. Philipp, I. Nehls, W. Wagenknecht, <sup>13</sup>C NMR spectroscopic study of the homogeneous sulphation of cellulose and xylan in the N<sub>2</sub>O<sub>4</sub> DMF system, *Carbohydr. Res.* 164 (1987) 107–116.
- [135] L.A. Ramos, E. Frollini, T. Heinze, Carboxymethylation of cellulose in the new solvent dimethyl sulfoxide/tetrabutylammonium fluoride, *Carbohydr. Polym.* 60 (2005) 259–267.
- [136] T. Heinze, R. Dicke, A. Koschella, A.H. Kull, E.A. Klotz, W. Koch, Effective preparation of cellulose derivatives in a new simple cellulose solvent, *Macromol. Chem. Phys.* 201 (2000) 627–631.
- [137] T. Heinze, T. Liebert, Unconventional methods in cellulose functionalization, *Prog. Polym. Sci.* 26 (2001) 1689–1762.
- [138] H. Harms, Lenzing LYOCCELL – chances of a new generation of manmade fibres, *Materialwiss. Werkstofftech.* 34 (2003) 267–271.
- [139] S.K. Lim, K.M. Cho, S. Tasaka, N. Inagaki, Mesoporous carbon fibers prepared from regenerated rice straw fibers, *Macromol. Mater. Eng.* 286 (2001) 187–190.
- [140] S.K. Lim, T.W. Son, D.W. Lee, B.K. Park, K.M. Cho, Novel regenerated cellulose fibers from rice straw, *J. Appl. Polym. Sci.* 82 (2001) 1705–1708.
- [141] M.B. Turner, S.K. Spear, J.D. Holbrey, R.D. Rogers, Production of bioactive cellulose films reconstituted from ionic liquids, *Biomacromolecules* 5 (2004) 1379–1384.
- [142] R.P. Swatloski, J.D. Holbrey, J.L. Weston, R.D. Rogers, Preparation of magnetic cellulose composites using ionic liquids, *Chim. Oggi* 24 (2006) 31–35.
- [143] M.B. Turner, S.K. Spear, J.D. Holbrey, D.T. Daly, R.D. Rogers, Ionic liquid reconstituted cellulose composites as solid support matrices for biocatalyst immobilization, *Biomacromolecules* 6 (2005) 2497–2502.
- [144] M.B. Turner, S.K. Spear, J.G. Huddleston, J.D. Holbrey, R.D. Rogers, Ionic liquid salt induced inactivation and unfolding of cellulase from *Trichoderma reesei*, *Green Chem.* 5 (2003) 443–447.
- [145] R.D. Rogers, J.D. Holbrey, S.K. Spear, M.B. Turner, Ionic liquids as green solvents: engineering bioactive cellulose materials, *Abstr. Pap. Am. Chem. Soc.* 227 (2004) U310.
- [146] O.A. El Seoud, A. Koschella, L.C. Fidale, S. Dorn, T. Heinze, Applications of ionic liquids in carbohydrate chemistry: a window of opportunities, *Biomacromolecules* 8 (2007) 2629–2647.
- [147] H.B. Xie, S.H. Li, S.B. Zhang, Ionic liquids as novel solvents for the dissolution and blending of wool keratin fibers, *Green Chem.* 7 (2005) 606–608.
- [148] V.M. Egorov, S.V. Smirnova, A.A. Formanovsky, I.V. Pletnev, Y.A. Zolotov, Dissolution of cellulose in ionic liquids as a way to obtain test materials for metal ion detection, *Anal. Bioanal. Chem.* 387 (2007) 2263–2269.
- [149] J.I. Kadokawa, M.A. Murakami, Y. Kaneko, A facile method for preparation of composites composed of cellulose and a polystyrene type polymeric ionic liquid using a polymerizable ionic liquid, *Compos. Sci. Technol.* 68 (2008) 493–498.
- [150] J.I. Kadokawa, M.A. Murakami, Y. Kaneko, A facile preparation of gel materials from a solution of cellulose in ionic liquid, *Carbohydr. Res.* 343 (2008) 769–772.
- [151] M.A. Murakami, Y. Kaneko, J.I. Kadokawa, Preparation of cellulose polymerized ionic liquid composite by in situ polymerization of polymerizable ionic liquid in cellulose dissolving solution, *Carbohydr. Polym.* 69 (2007) 378–381.
- [152] C. Tsiptsias, C. Panayiotou, Preparation of cellulose nanohydroxyapatite composite scaffolds from ionic liquid solutions, *Carbohydr. Polym.* 74 (2008) 99–105.
- [153] C. Tsiptsias, A. Stefopoulos, I. Kokkinomalis, L. Papadopoulou, C. Panayiotou, Development of micro and nano porous composite materials by processing cellulose with ionic liquids and supercritical CO<sub>2</sub>, *Green Chem.* 10 (2008) 965–971.
- [154] A.P. Dadi, C.A. Schall, S. Varanasi, Mitigation of cellulose recalcitrance to enzymatic hydrolysis by ionic liquid pretreatment, *Appl. Biochem. Biotechnol.* 137 (2007) 407–421.
- [155] A.P. Dadi, S. Varanasi, C.A. Schall, Enhancement of cellulose saccharification kinetics using an ionic liquid pretreatment step, *Biotechnol. Bioeng.* 95 (2006) 904–910.
- [156] H. Zhao, C.I.L. Jones, G.A. Baker, S. Xia, O. Olubajo, V.N. Person, Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis, *J. Biotechnol.* 139 (2009) 47–54.
- [157] N. Kamiya, Y. Matsushita, M. Hanaki, K. Nakashima, M. Narita, M. Goto, H. Takahashi, Enzymatic in situ saccharification of cellulose in aqueous ionic liquid media, *Biotechnol. Lett.* 30 (2008) 1037–1040.
- [158] C.Z. Li, Q. Wang, Z.K. Zhao, Acid in ionic liquid: an efficient system for hydrolysis of lignocellulose, *Green Chem.* 10 (2008) 177–182.
- [159] C.Z. Li, Z.K.B. Zhao, Efficient acid catalyzed hydrolysis of cellulose in ionic liquid, *Adv. Synth. Catal.* 349 (2007) 1847–1850.
- [160] O.A. El Seoud, G.A. Marson, G.T. Giacco, E. Frollini, An efficient, one pot acylation of cellulose under homogeneous reaction conditions, *Macromol. Chem. Phys.* 201 (2000) 882–889.
- [161] A.M. Regiani, E. Frollini, G.A. Marson, G.M. Arantes, O.A. El Seoud, Some aspects of acylation of cellulose under homogeneous solution conditions, *J. Polym. Sci. Polym. Chem.* 37 (1999) 1357–1363.
- [162] G.A. Marson, O.A. El Seoud, A novel, efficient procedure for acylation of cellulose under homogeneous solution conditions, *J. Appl. Polym. Sci.* 74 (1999) 1355–1360.



- [163] M.A. Hussain, T. Liebert, T. Heinze, Acylation of cellulose with N,N'-carbonyldiimidazole activated acids in the novel solvent dimethyl sulfoxide/tetrabutylammonium fluoride, *Macromol. Rapid Commun.* 25 (2004) 916–920.
- [164] S. Fischer, K. Thummler, K. Pfeiffer, T. Liebert, T. Heinze, Evaluation of molten inorganic salt hydrates as reaction medium for the derivatization of cellulose, *Cellulose* 9 (2002) 293–300.
- [165] A. Biswas, R.L. Shogren, J.L. Willett, Solvent free process to esterify polysaccharides, *Biomacromolecules* 6 (2005) 1843–1845.
- [166] K.J. Edgar, C.M. Buchanan, J.S. Debenham, P.A. Rundquist, B.D. Seiler, M.C. Shelton, D. Tindall, Advances in cellulose ester performance and application, *Prog. Polym. Sci.* 26 (2001) 1605–1688.
- [167] A. Biswas, G. Selling, M. Appell, K.K. Woods, J.L. Willett, C.M. Buchanan, Iodine catalyzed esterification of cellulose using reduced levels of solvent, *Carbohydr. Polym.* 68 (2007) 555–560.
- [168] A. Hummel, Acetate manufacturing, process and technology 3.2 industrial processes, *Macromol. Symp.* 208 (2003) 61–79.
- [169] A. Biswas, B.C. Saha, J.W. Lawton, R.L. Shogren, J.L. Willett, Process for obtaining cellulose acetate from agricultural by products, *Carbohydr. Polym.* 64 (2006) 134–137.
- [170] D. de Maria, A. Martinsson, Ionic liquid based method to determine the degree of esterification in cellulose fibers, *Analyst* 134 (2009) 493–496.
- [171] T. Heinze, T.F. Liebert, K.S. Pfeiffer, M.A. Hussain, Unconventional cellulose esters: synthesis, characterization and structure property relations, *Cellulose* 10 (2003) 283–296.
- [172] J. Wu, H. Zhang, J. Zhang, J.S. He, Homogeneous acetylation and regioselectivity of cellulose in a new ionic liquid, *Chem. J. Chin. Univ.* 27 (2006) 592–594.
- [173] J. Wu, J. Zhang, H. Zhang, J.S. He, Q. Ren, M. Guo, Homogeneous acetylation of cellulose in a new ionic liquid, *Biomacromolecules* 5 (2004) 266–268.
- [174] Y. Cao, J. Wu, T. Meng, J. Zhang, J.S. He, H.Q. Li, Y. Zhang, Acetone soluble cellulose acetates prepared by one step homogeneous acetylation of cornhusk cellulose in an ionic liquid 1-allyl-3-methylimidazolium chloride (AmimCl), *Carbohydr. Polym.* 69 (2007) 665–672.
- [175] S. Barthel, T. Heinze, Acylation and carbanilation of cellulose in ionic liquids, *Green Chem.* 8 (2006) 301–306.
- [176] H. Leipner, S. Fischer, E. Brendler, W. Voigt, Structural changes of cellulose dissolved in molten salt hydrates, *Macromol. Chem. Phys.* 201 (2000) 2041–2049.
- [177] S. Fischer, H. Leipner, T. Liebert, T. Heinze, An alternative approach for deprotection of triphenylmethyl cellulose, *Polym. Bull.* 45 (2001) 517–522.
- [178] T. Yoshimura, K. Matsuo, R. Fujioka, Novel biodegradable superabsorbent hydrogels derived from cotton cellulose and succinic anhydride: synthesis and characterization, *J. Appl. Polym. Sci.* 99 (2006) 3251–3256.
- [179] S. Hadano, K. Onimura, H. Tsutsumi, H. Yamasaki, T. Oishi, Syntheses of chemical modified cellulose obtained from waste pulp, *J. Appl. Polym. Sci.* 90 (2003) 2059–2065.
- [180] C.F. Liu, R.C. Sun, A.P. Zhang, J.L. Ren, Z.C. Geng, Structural and thermal characterization of sugarcane bagasse cellulose succinates prepared in ionic liquid, *Polym. Degrad. Stab.* 91 (2006) 3040–3047.
- [181] C.F. Liu, R.C. Sun, A.P. Zhang, J.L. Ren, X.A. Wang, M.H. Qin, Z.N. Chao, W. Luo, Homogeneous modification of sugarcane bagasse cellulose with succinic anhydride using a ionic liquid as reaction medium, *Carbohydr. Res.* 342 (2007) 919–926.
- [182] C.F. Liu, R.C. Sun, A.P. Zhang, J.L. Ren, Preparation of sugarcane bagasse cellulosic phthalate using an ionic liquid as reaction medium, *Carbohydr. Polym.* 68 (2007) 17–25.
- [183] C.F. Liu, R.C. Sun, A.P. Zhang, M.H. Qin, J.L. Ren, X.A. Wang, Preparation and characterization of phthalated cellulose derivatives in room temperature ionic liquid without catalysts, *J. Agric. Food Chem.* 55 (2007) 2399–2406.
- [184] C.F. Liu, A.P. Zhang, W.Y. Li, F.X. Yue, R.C. Sun, Homogeneous modification of cellulose in ionic liquid with succinic anhydride using N-bromosuccinimide as a catalyst, *J. Agric. Food Chem.* 57 (2009) 1814–1820.
- [185] J.L. Ren, R.C. Sun, F. Peng, Carboxymethylation of hemicelluloses isolated from sugarcane bagasse, *Polym. Degrad. Stab.* 93 (2008) 786–793.
- [186] T. Heinze, T. Liebert, P. Klufers, F. Meister, Carboxymethylation of cellulose in unconventional media, *Cellulose* 6 (1999) 153–165.
- [187] Y. Tezuka, Y. Tsuchiya, T. Shiomi, <sup>13</sup>C NMR determination of substituent distribution in carboxymethylcellulose by use of its peresterified derivatives, *Carbohydr. Res.* 291 (1996) 99–108.
- [188] T. Heinze, K. Pfeiffer, Studies on the synthesis and characterization of carboxymethylcellulose, *Angew. Makromol. Chem.* 266 (1999) 37–45.
- [189] M. Granstrom, J. Kavakka, A. King, J. Majoinen, V. Makela, J. Helaja, S. Hietala, T. Virtanen, S.L. Maunu, D.S. Argyropoulos, I. Kilpelainen, Tosylation and acylation of cellulose in 1-allyl-3-methylimidazolium chloride, *Cellulose* 15 (2008) 481–488.

# Lignin

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## 6.1 INTRODUCTION

Lignin, as a natural polymer, exists in all terrestrial plants, although some aquatic organisms may contain lignin or “lignin-like” components [1, 2]. It is well accepted that lignin is a phenolic polymer derived primarily from three hydroxycinnamyl alcohols or monolignols, namely, *p*-coumaryl alcohol ( $M_H$ ), coniferyl alcohol ( $M_G$ ), and sinapyl alcohol ( $M_S$ ) (Fig. 6.1) by free radical generation, followed by chemical coupling processes. Lignin molecules, starting with dimerization of two monolignol radicals, grow via cross-coupling between a monolignol radical and the previously formed dimeric or oligomeric lignol radicals [3–5]. Although lignin has been studied for more than a century and much progress has been made regarding the biosynthesis of monolignols and structural elucidation of lignins, controversies or debates still exist in terms of lignin’s structural regularity and the lignification process [6–9].

The term “lignin” (Latin *lignum* = wood) was put forward in 1865 by Schulze to describe the dissolved part of wood when treated with nitric acid [10, 11]. In the 1960s, when powerful modern analytical tools of biochemistry and organic chemistry were applied, interesting information became to accumulate. Since then lignin research has proceeded at a fast pace and has attracted much attention because of the dominant pulping industries [12]. However, the definition of lignin has never been as clear as that of other natural polymers such as cellulose and protein because of the extreme complexity that affects its isolation, compositional analysis, and structural characterization.

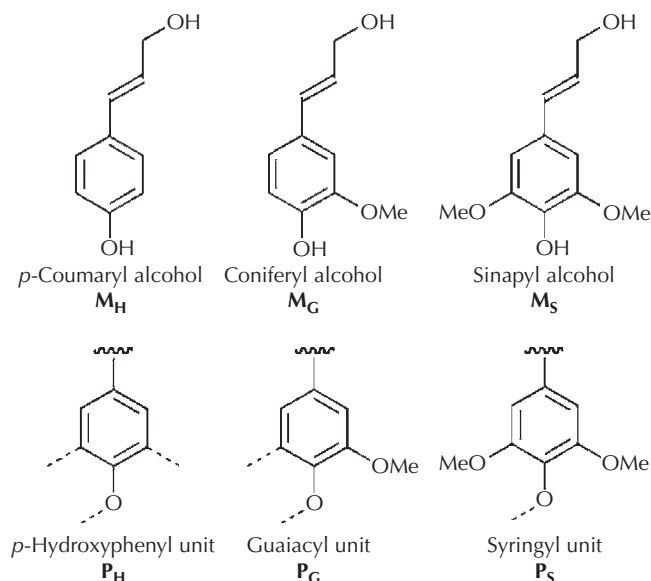
Many attempts have been made to define lignin or lignins based on the constitution, structural features, and mechanism of formation. The problem of lack of precise definition for lignin is associated with its nature: no regularly repeating multiunit structures have been found, and compositions and structures of lignin vary depending on their origins. Any simplified definition would risk of excluding aspects of lignin. The definition given by Brauns

was thought to be generally accepted [13]. By this definition, lignins have the following characteristics:

1. Lignins are plant polymers made from phenylpropanoid building units;
2. Lignins contain most of the wood methoxyl content;
3. Lignins are resistant to acid hydrolysis, readily oxidized, soluble in hot alkaline and bisulfite, and readily condensed with phenols or thiols;
4. When reacted with nitrobenzene in hot alkaline solution, lignins yield mainly vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde depending on the origin of the lignins;
5. When boiled in ethanolic hydrochloric acid solution, lignins give “Hibbert’s ketone” monomers, a mixture of aromatic ketones resulting from cleavage of lignins’ major inter-unit ether ( $\beta$ -O-4) linkages.

In a recent review of lignins, a half page long definition given by Brunow was cited as the most concise and comprehensive one to date [8]. This summary description of lignins is based on knowledge available today. By this definition, lignins or protolignins have the following features:

1. Protolignins are biopolymers consisting of phenylpropanoid units with an oxygen atom at the *p*-position (as HO or O–C) and with none, one or two methoxyl groups in the *o*-positions to this oxygen atom.
2. The phenylpropanoid building units are connected to one another by a series of characteristic linkages ( $\beta$ -O-4,  $\beta$ -5,  $\beta$ - $\beta$ , etc.). There are a series of characteristic end groups (e.g. cinnamaldehyde units) that exist in lignins.
3. All the types of structural elements detected in protolignins are consistent with those formed by oxidation of the *p*-hydroxycinnamyl alcohols in vitro. The structural units in protolignins are not linked to one another in any particular order. Lignins are not optically active.
4. Protolignins are branched and cross-linked to other cell wall components. There are strong indications of the



**FIGURE 6.1** The three primary lignin monomers  $M$ , monolignols *p* coumaryl alcohol  $M_H$ , coniferyl alcohol  $M_G$ , and sinapyl alcohol  $M_S$  and lignin structural *p* hydroxyphenyl, guaiacyl, and syringyl units  $P_H$ ,  $P_G$ , and  $P_S$ , derived from them through free radical coupling reactions. The carbon atom numbering follows the traditional convention for lignin. (Adapted from Ralph et al. [8]).

occurrence of linkages between lignins and carbohydrates. There are esters that exist in some types of lignins e.g. grass lignins with *p*-coumarates and aspen lignin with *p*-hydroxybenzoates.

From these definitions of lignin it is very clear that lignin, never defined as a compound, is a class of phenolic natural polymers with broad compositions and a variety of linkages between building units. There are many excellent book chapters and review articles that cover various aspects of lignins (structure, composition, and biosynthesis), mainly wood lignins [5–10, 14–22]. A recent review by Buranov and Mazza covers some structural characteristics and isolation processes for lignins from herbaceous crops including corn, wheat, rice, and flax straw [23]. However, some issues about lignins in grass plants still need to be addressed. Here, we will discuss some crucial aspects including monolignol synthesis, lignification, isolation, analytical methods, and structural characteristics of grass lignins. The utilization of lignins, in general, will be also addressed briefly at the end of this chapter.

## 6.2 MONOLIGNOLS, AND LIGNIFICATION IN GRASS CELL WALLS

Most studies on lignin biosynthesis have been conducted with woody dicot or gymnosperm species because of the major interest in reducing the cost and environmental impact of removing lignin from woody plants during pulping for paper production [16]. However, lignins in

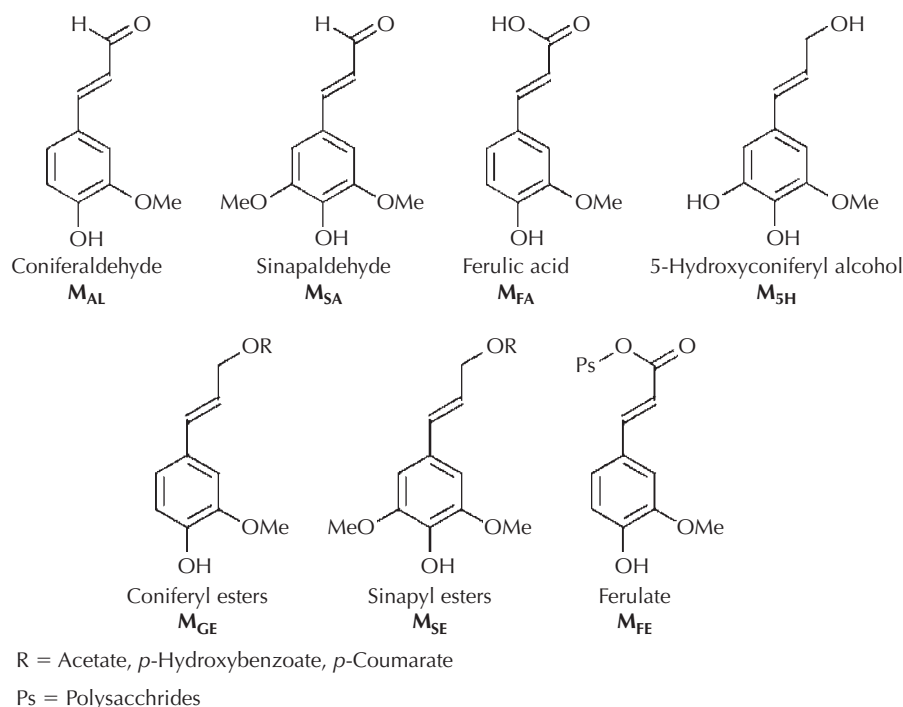
herbaceous plants are receiving increasing attention not only due to their annual renewability and vast availability (1549 million tons/year worldwide) [24] but also to their crucial roles in efficient utilization of herbaceous biomass such as forage grasses, agricultural residues such as cereal straws, and bioenergy crops [25, 26].

Like other polymers, lignins are polymerized or assembled from their monomers, so-called monolignols. It had been thought that lignins have three monomers, namely *p*-coumaryl alcohol ( $M_H$ ), coniferyl alcohol ( $M_G$ ), and sinapyl alcohol ( $M_S$ ) (Fig. 6.1). However, thanks to advanced analytical tools developed and applied in studies on biosynthesis of lignin monomers, it becomes clear that lignins contain more monomers than those three traditional ones. For example, coniferaldehyde ( $M_{AL}$ ), 5-OH coniferyl alcohol ( $M_{SH}$ ), and acylated monolignols ( $M_{GE}$  and  $M_{SE}$ ) (Fig. 6.2) have been demonstrated to incorporate themselves into lignins via free radical coupling reactions in some plants [27–35]. They should be considered as authentic lignin monomers.

### 6.2.1 Biosynthesis of Monolignols

The biosynthetic pathway for monolignols has been studied intensively and many genes encoding enzymes involved in every step leading to the monolignols have been identified [7, 36]. Proposals for the favored pathways through the metabolic grid have been reviewed [7, 34]. Although lignins in herbaceous plants are different from those in trees in terms of composition and, structural features, large parts of biosynthetic pathways to the three main monolignols are in common (Fig. 6.3).

The first step in monolignol biosynthesis is the deamination of L-phenylalanine or tyrosine by phenylalanine ammonia-lyase (PAL) or tyrosine ammonia-lyase (TAL), yielding cinnamic or *p*-coumaric acids (*p*-CA), respectively. Hydroxylation at the C4 position of cinnamic acid to *p*-CA is catalyzed by cinnamic acid 4-hydroxylase (C4H), a cytochrome P450 monooxygenase belonging to the CYP73 subfamily [37]. The formation of hydroxycinnamoyl-CoA esters involved in the biosynthesis of a wide variety of phenolics is catalyzed by 4-coumarate: CoA ligase (4CL). Reduction of hydroxycinnamoyl-CoA thioesters to the corresponding hydroxycinnamaldehydes is catalyzed by cinnamoyl-CoA reductase (CCR). Cinnamyl alcohol dehydrogenase (CAD) catalyzes the last step in the biosynthesis of the lignin precursors, which is the reduction of cinnamaldehydes to cinnamyl alcohols. *p*-Coumarate 3-hydroxylase (C3H) was long thought to catalyze the hydroxylation at the C3 position of *p*-CA to form caffeic acid. However, C3H has only recently been cloned from Arabidopsis and shown to be a cytochrome P<sub>450</sub>-dependent monooxygenase [38, 39]. Enzymatic assays demonstrated that the shikimate and quinate esters of *p*-CA are the



**FIGURE 6.2** Recent studies have demonstrated that various plants including grasses can use other monomers, including those shown here, as lignin precursors, to produce lignins with new structures. (Modified from Vanholme et al. [34]).

preferred substrates for C3H [38–40]. Therefore, along monolignol biosynthesis pathway, at least in *Arabidopsis*, *p*-coumarate should be first converted to *p*-coumaroyl-CoA by 4CL followed by an acyltransferase-catalyzed conversion to *p*-coumaroyl shikimate and *p*-coumaroyl quinate, the substrates for C3H. These acyltransferase enzymes have been described as reversible enzymes that can also convert caffeoyl-shikimate or caffeoyl-quininate (chlorogenic acid) into caffeoyl-CoA, the substrate for CCoAOMT [39–41]. Recently, *p*-hydroxycinnamoyl-CoA: quinate shikimate *p*-hydroxycinnamoyltransferase (HCT), a reversible acyltransferase with such capability, has been purified and the corresponding gene cloned from tobacco [42]. The predominant role of CCR is to catalyze the reduction of hydroxycinnamoyl-CoA thioesters to the corresponding aldehydes. CCoAOMT methylates caffeoyl-CoA to produce feruloyl-CoA. Ferulate 5-hydroxylase (F5H) was first found to catalyze the hydroxylation at the C5 position of ferulic acid to 5-OH-ferulic acid. However, it was found recently that the preferential substrate for F5H was not ferulic acid, but coniferaldehyde and coniferyl alcohol [43, 44]. Similarly, caffeic acid *O*-methyltransferase (COMT) was thought to methylate caffeic acid/5-OH-ferulic acid into ferulic acid/sinapic acid. Actually, the products of F5H-catalyzed hydroxylation, 5-hydroxyconiferaldehyde, and 5-hydroxyconiferyl alcohol proved to be good substrates for COMT, whereas caffeic acid was a poor substrate [43–46].

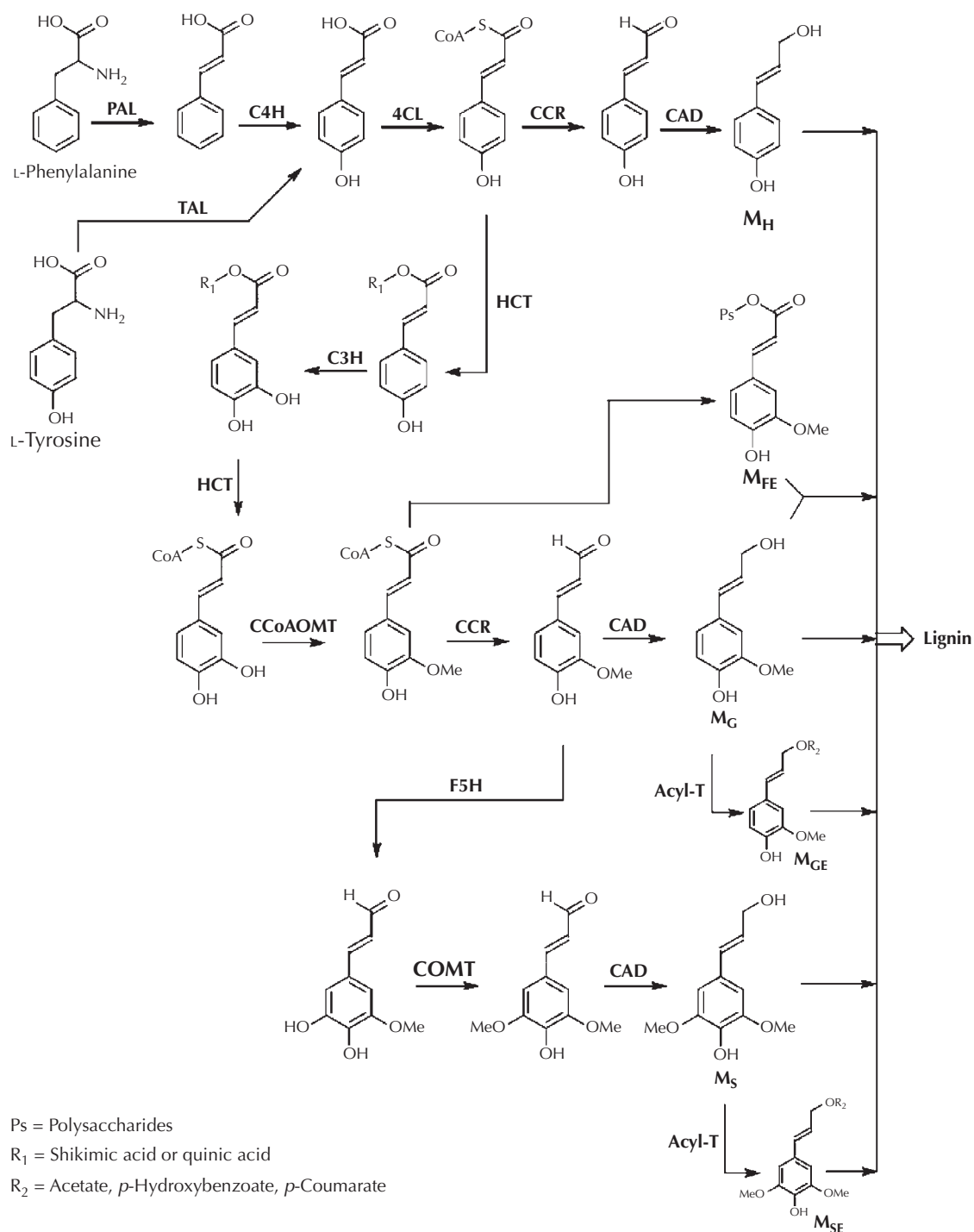
We have demonstrated that grass lignins derive not just from the three traditional monolignols but also from

acylated sinapyl/coniferyl alcohols. These, as well as ferulates, should also be considered as precursors of grass lignins [32–34, 47]. In grass cell walls, at least in corn, there are acyltransferases that catalyze acylation of sinapyl/coniferyl alcohols to produce acylated conjugates (Hatfield et al. unpublished). Those acylated monolignols and ferulates readily copolymerize with *p*-coumaryl, coniferyl, and sinapyl alcohols to produce grass lignins with unique structural features (Fig. 6.3).

### 6.2.2 Lignin Deposition and Topochemistry

Once synthesized in the cytoplasm, monolignols are transported (perhaps as monolignol glucosides in some plants) to the cell wall where they are oxidized and polymerized to form lignins comprised of two major unit types – guaiacyl ( $P_G$ ), derived from coniferyl alcohol and syringyl ( $P_S$ ), derived from sinapyl alcohol. *p*-Hydroxyphenyl units ( $P_H$ ), derived from *p*-coumaryl alcohol, occur only as a minor component of lignin except in softwood compression wood regions or in some transgenics [48].

Lignin deposition is one of the final stages of xylem cell differentiation and mainly takes place during secondary thickening of the cell wall. Lignification starts at the cell corners in the region of the middle lamella and the primary wall. Normally, lignification proceeds from primary to secondary cell walls in forage grasses and legumes [49–51], but the reverse was observed for sclerenchyma in alfalfa [52].



**FIGURE 6.3** Biosynthetic pathways to monolignols in grasses. PAL, phenylalanine ammonia lyase; TAL, tyrosine ammonia lyase; C4H, cinnamate 4 hydroxylase; 4CL, 4 coumarate: CoA ligase; HCT, *p* hydroxycinnamoyl CoA: quinate shikimate *p* hydroxycinnamoyltransferase; C3H, *p* coumarate 3 hydroxylase; CCoAOMT, caffeoyl CoA *O* methyltransferase; CCR, cinnamoyl CoA reductase; COMT, caffeic acid/5 hydroxyconifer aldehyde *O* methyltransferase; CAD, cinnamyl alcohol dehydrogenase; F5H, ferulate 5 hydroxylase; Acyl T, acyltransferase (Adapted from Vanholme et al. [34]).

Generally, lignin concentration is higher in the middle lamella and cell corners than in the S<sub>2</sub> secondary wall [36, 48, 53]. However, because it occupies a larger portion of the wall, most of the lignin is in the secondary wall. Microautoradiography and ultraviolet (UV)-microspectrometric studies by Terashima

and coworkers [49], indicated that the incorporation of the three monolignols in grass lignins is spatially and temporally regulated and varies between primary and secondary cell walls and among tissues. The incorporation of P<sub>H</sub> and P<sub>G</sub> units takes place at the onset of lignification



in cell corners and the middle lamellae. Syringyl lignins, however, have been detected in immature maize coleoptiles, suggesting that their deposition also begins at the early stages of lignification [54]. Using immunological probes coupled with transmission electron microscopy (TEM) allowed visualization of the progression of lignin deposition during maturation in each cell type, and characterization of structural variations in the lignin macromolecule. Results from these immunocytochemistry studies on maize cell walls showed that all tissues neither undergo lignification simultaneously nor at the same rate, indicating that lignification is a spatial and temporal phenomenon, which varies according to tissues and cell walls [55, 56]. In gramineous monocotyledons, lignin incorporates significant amounts of hydroxycinnamate esters. Ferulate-polysaccharide esters  $M_{FA}$  (and minor amounts of the *p*-coumarate analogs  $M_{PCA}$ ) are rapidly deposited at the early stages of lignification. *p*-Coumarates, acylating lignin sidechains mainly on  $P_S$  units [57], are deposited throughout lignification [58], implicating the involvement of acylated monomers  $M_{GE}$  and  $M_{SE}$ .

### 6.2.3 Lignification Polymerization of Monolignols

Lignification is a polymerization process in which lignin macromolecules grow from monomers via free radical coupling mechanisms. Lignification starts with dehydrogenative dimerization or cross-dimerization of two monolignol molecules and continues with further cross-coupling of the preformed dimers or oligomers and incoming monolignols so that lignin macromolecules are produced [59]. Cross-coupling between two growing lignin macromolecules may also occur when a free-phenolic guaiacyl unit is available for free radical coupling with another free-phenolic guaiacyl or syringyl unit. This cross-coupling between two growing lignin polymers produces 5-5- or 4-*O*-5-linkages that have not been demonstrated to be formed between two monolignols [8] (Fig. 6.4).

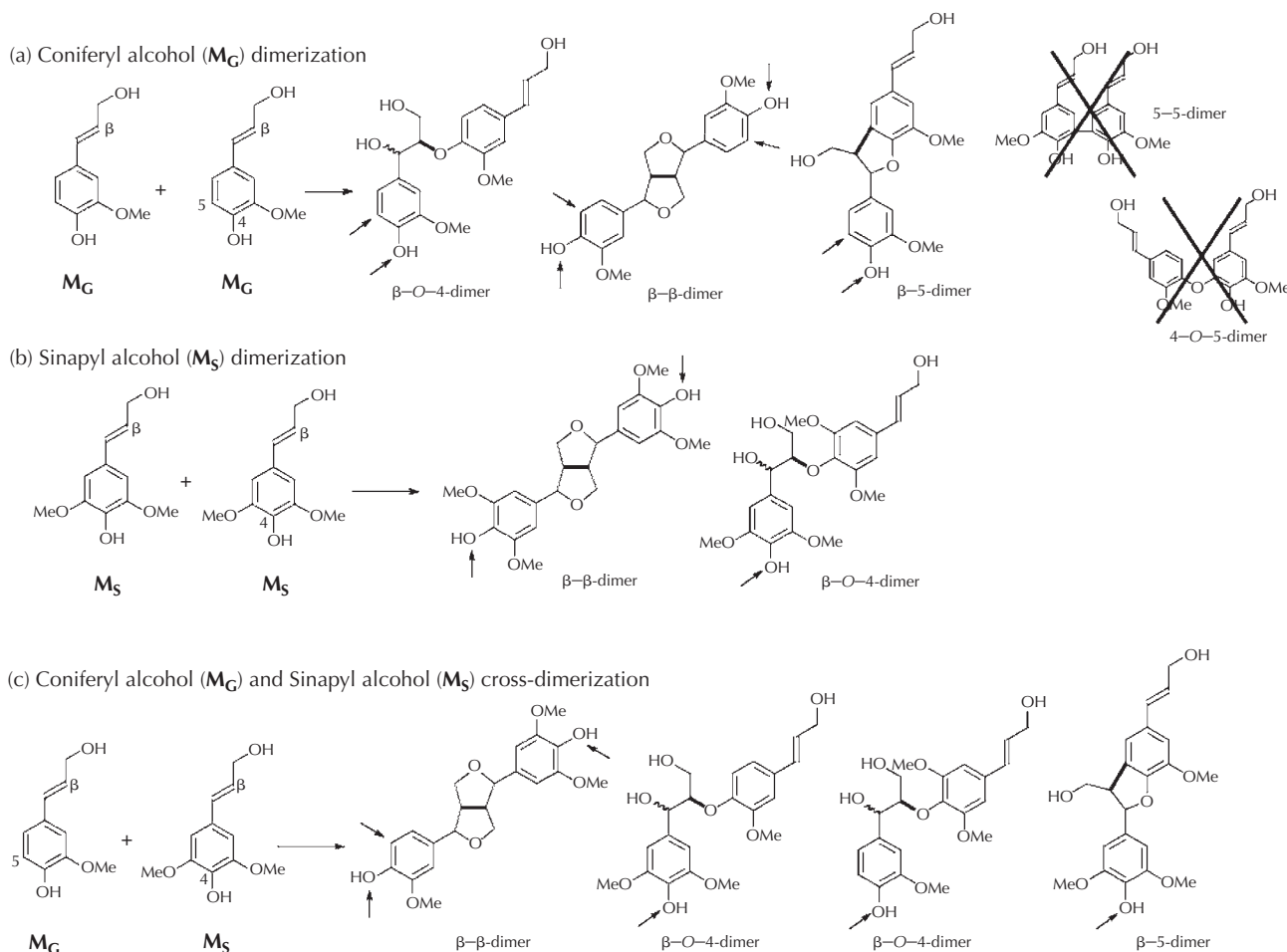
Therefore, the first action on lignin monomers in lignification is dehydrogenation. The enzymatic dehydrogenation of a monolignol is a one-electron transfer producing a phenoxy radical stabilized by resonance. Dehydrodimerization of monolignols results in up to three kinds of linkages:  $\beta$ -*O*-4,  $\beta$ -5, and  $\beta$ - $\beta$  [60–62]. For coniferyl alcohol ( $M_G$ ), these dimers are  $\beta$ -ether units, phenylcoumarans, or resinols. *p*-Coumaryl alcohol also dimerizes producing three dimers with similar couple modes to  $M_G$ , whereas only  $\beta$ -*O*-4- and  $\beta$ - $\beta$ -coupled dimers are formed from sinapyl alcohol ( $M_S$ ) dimerization [63]. The 5-5- and 4-*O*-5-dehydrodimers shown in many texts (and crossed out in Fig. 6.4) do not arise in any significant way from monolignol dehydrodimerization reactions; monolignol coupling involves the  $\beta$ -position of at least

one of the monolignols. Subject to simple chemical compatibility, cross-coupling between monolignols can also occur giving mixed G/S analogs (Fig. 6.4).

One feature of dehydrodimerization reactions is the formation of resinol ( $\beta$ - $\beta$ -coupled) structures and a cinnamyl end group from  $\beta$ -*O*-4- or  $\beta$ -5-dehydrodimerization. Actually, there are very limited numbers of these cinnamyl end groups in lignins implying that the most significant reactions are cross-couplings of a new incoming monomer (radical) with the preformed dimers, oligomers, and the growing polymers [15, 64]. This process has been characterized as “endwise” polymerization as illustrated in Fig. 6.5. The “endwise” polymerization produces a linear lignin macromolecule if the noncycle benzyl aryl ethers, presumably formed through nucleophilic addition of phenols to the quinone methides intermediates, are ignored; such products are insignificant in most lignins analyzed to date. However, branching is possible during lignification when two preformed or growing lignin polymers couple each other through 5-5- or 4-*O*-5-coupling mode (Fig. 6.6).

In grass plants, the involvement of ferulates/diferulates and acylated monolignols in the lignification process makes lignins, in these plants, special in terms of their lignin structures and alkaline solubility, as we will discuss later in this chapter. Ferulates, feruloyl polysaccharides, readily cross-couple with monolignols producing cross-dimers with various linkages, as demonstrated in a model study [65] (Fig. 6.7). Cross-dimers from ferulates and coniferyl alcohol have been identified in alkaline hydrolysates from various grass cell walls [66, 67]. These cross-dimers formed from ferulates and monolignols are able to couple with a new monolignol, extending the polymer. As these processes continue, lignin–hydroxycinnamate–carbohydrate complexes result [68]. Thus, lignins are cross-linked to carbohydrates by ferulate/diferulate through radical cross-coupling reactions that produce various linkages between ferulates and lignin, of which only the 4-*O*- $\beta$  bond can be cleaved by current solvolytic methods [69] (Fig. 6.8). Lignin–polysaccharide cross-linking mediated by ferulates is thought to be responsible for the limited digestibility of mature grass cell wall by ruminal animals [70, 71]. However, an alkaline treatment, which cleaves all esters in grass cell walls breaking down all cross-linkages between polysaccharides and lignin, significantly releases lignin from cell walls and increases the cell wall degradability by ruminal bacteria or cellulolytic enzymes [25, 72, 73].

Acylated monolignols are able to participate in lignification, as demonstrated in kenaf in which acetylated sinapyl alcohol is incorporated as an authentic monolignol into lignin producing a unique aryl-tetrahydrofuran structure [32]. Similar structures have been revealed in grass lignins by nuclear magnetic resonance spectrometry (NMR) [33]. Model studies with sinapyl *p*-coumarate indicated that these tetrahydrofuran structures result from



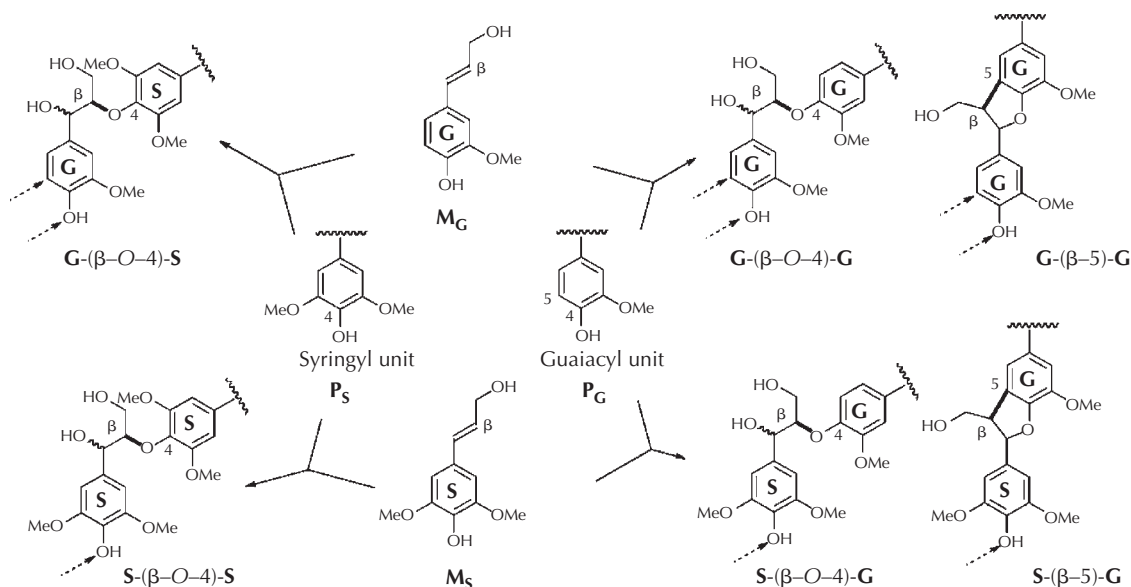
**FIGURE 6.4** Dehydrodimerization of monolignols. (a) Dehydrodimerization of coniferyl alcohol  $M_G$  produces only three dimers, in which at least one is coupled at its  $\beta$  position. The 5 5 and 4 O 5 dehydrodimers do not actually arise in any significant way from monolignol dimerizations. (b) Dehydro dimerization of sinapyl alcohol  $M_S$  produces  $\beta\beta$  and  $\beta O 4$  coupled dimers. (c) Cross dimerization of  $M_G$  and  $M_S$  produces four crossed dimers, G ( $\beta\beta$ ) S, S ( $\beta O 4$ ) G, G ( $\beta O 4$ ) S, and S ( $\beta\beta$ ) G. (Adapted from Ralph [8]).

dehydrogenative dimerization or cross-dimerization of sinapyl *p*-coumarate and sinapyl alcohol (Fig. 6.9). The interesting result from this study is that the *p*-coumarate moiety, despite having free phenol, does not participate in dimerization while sinapyl moiety dimerizes forming  $\beta$ - $\beta$ -coupled/cross-coupled products with tetrahydrofuran structures bearing *p*-coumarate as pendant groups. This is inconsistent with NMR observations from corn cell walls and has been ascribed as a result of radical transfer from *p*-coumarate to sinapyl alcohol or syringyl moieties [74].

### 6.3 ISOLATION AND PURIFICATION OF LIGNINS

It is desirable to have pure samples to work with when quantitative or even qualitative characterizations are performed. However, it is not a trivial or impossible to isolate a pure lignin preparation from plant cell walls,

especially from grass plants in which lignin is intimately associated with polysaccharides and hydroxycinnamates as we will discuss in other sections of this chapter. It has always been a challenging task to isolate a desirable lignin preparation for compositional and structural analyses although many procedures have been developed for this purpose [75–77]. For historical reasons, previously developed methods for isolating lignins are dedicated to wood materials although these methods have been applied to grass plants with limited success [77, 78]. Currently, the most used lignin preparations for structural studies are milled wood lignin (MWL) and cellulytic enzyme lignin (CEL). Recently, some lignin preparations isolated by industrial pulping processes such as soda, alkali-hydrogen peroxide, and organsolv have also been studied. We are not going to describe detailed procedures of all previously developed methods for isolation and purification of lignins, instead brief reviews of these lignin preparations will be followed by a discussion on purification of isolated lignins from plant cell walls or industrial processes.



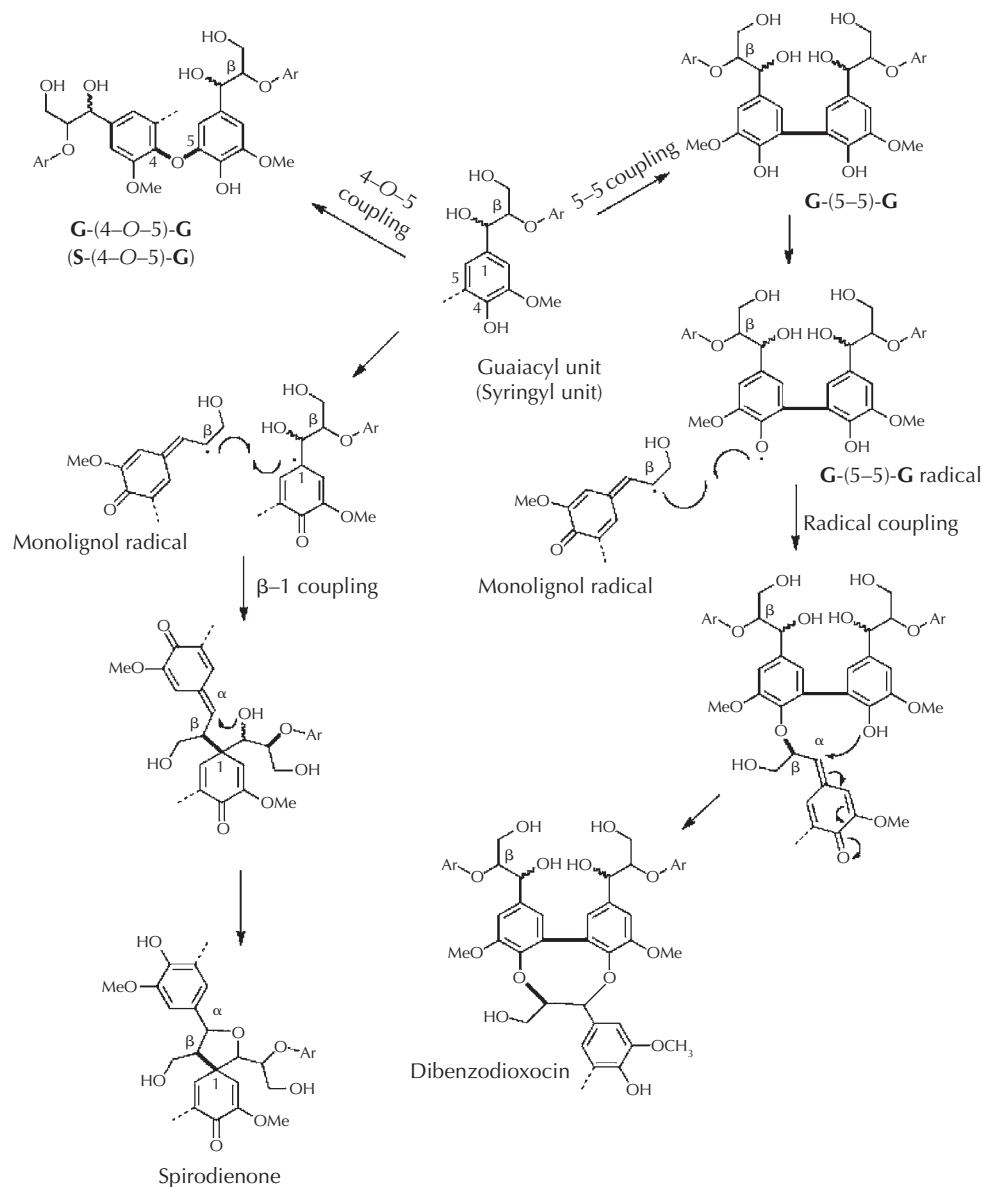
**FIGURE 6.5** Lignification proceeds by cross coupling of the monolignol  $M_G$  or  $M_S$  with a guaiacyl unit  $P_G$  or a syringyl unit  $P_S$ . Cross coupling between a  $P_G$  and a  $M_G$  or  $M_S$  gives two linkages,  $\beta O 4$  and  $\beta 5$  structures, whereas cross coupling between a  $P_S$  and a  $M_G$  or  $M_S$  produces only the  $\beta O 4$  coupled product. This explains why there are more  $\beta$  ethers in lignin than in dimerization of monolignols and why high S lignins have elevated  $\beta$  ether levels. A shorthand for naming units is introduced here, for example,  $G (\beta O 4) S$  units arise from coupling of a  $M_G$  at its  $\beta$  position with a syringyl phenolic end unit  $P_S$  of the growing polymer at its 4 O position. Sites of possible further coupling reactions during lignification are indicated by dashed arrows. (Adapted from Ralph et al. [8]).

### 6.3.1 Milled Wood Lignin

The first major advance toward separating lignin in a relatively unaltered state was made by Björkman [79], who developed a lignin separation procedure based on extensive grinding of plant material in a nonswelling liquid (toluene). Lignin in situ (proto lignin) is almost insoluble in any solvents unless it is degraded by physical or chemical treatments. Thus, ball milling is essential to isolate lignin from plant cell walls by solvent extractions. From ball-milled wood materials, lignin is ready to be extracted out by aqueous dioxane (usually with dioxane/water, 9/1, v/v). The isolated lignin obtained in this way is called MWL. The resulting MWL has largely been considered as a representative source of native lignin and has been extensively used in the elucidation of native lignin structure. However, for the Björkman procedure, concerns exist over the similarity between MWL and native lignin because of the low yields (25–50% of theoretical) and structural alterations because of ball milling [80]. It is believed that by increasing the extent of milling and, thus, MWL yield, a lignin sample more representative of the total lignin in wood is produced. Whiting and Goring [81] and Terashima et al. [82] further showed that MWL is not representative of the whole lignin in wood, but primarily originates in the secondary wall of the cell, with differences in rate of extraction after ball milling because of inherent differences in the chemistry of lignin in the middle lamella and the secondary wall being responsible.

Although it is evident that structures of lignin are modified in some extent by ball milling MWL has been proven to be the best preparation for structural studies of lignins, at least for wood samples [83].

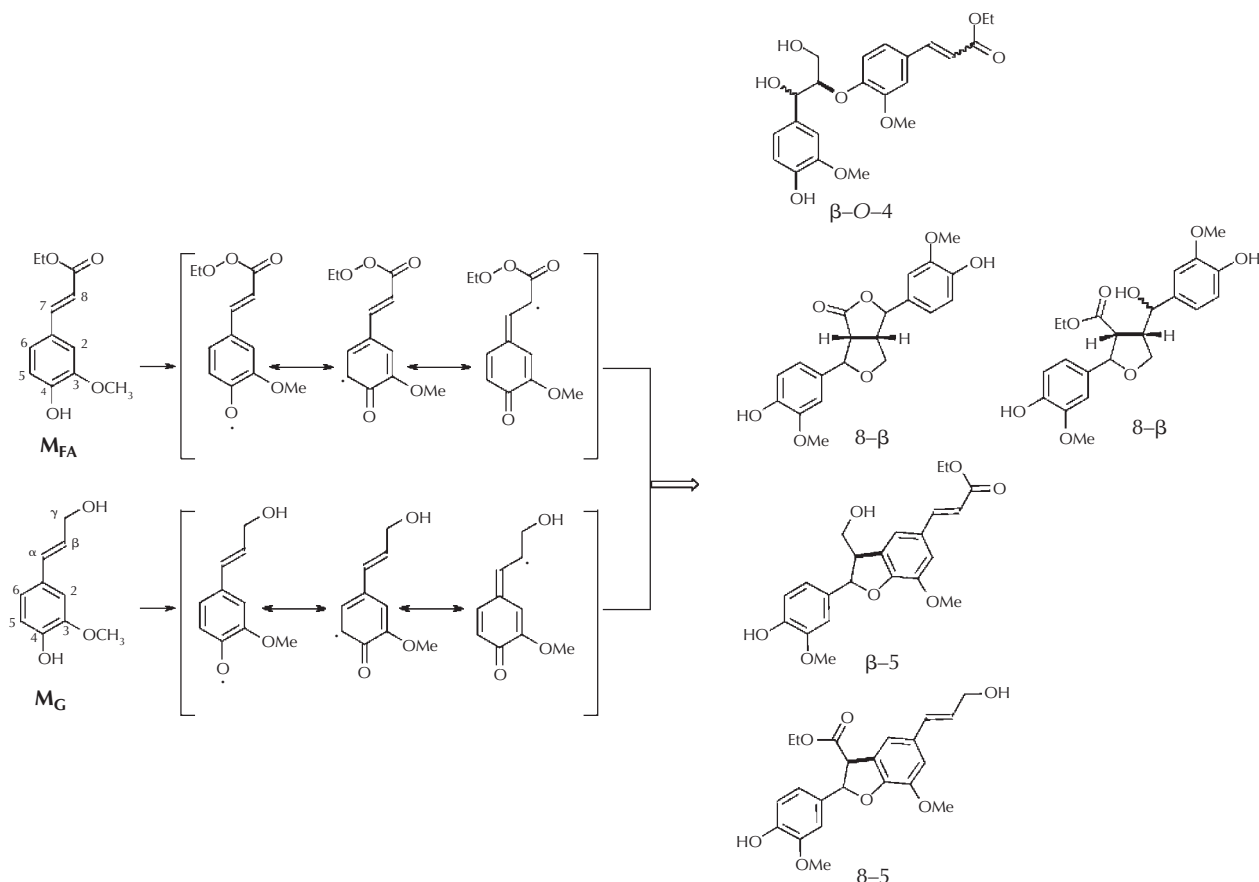
In the course towards improving the yields of isolated lignin while preventing the lignin preparation from too much damage caused by ball milling, many efforts have been made to understand the effects of ball milling and to improve the yield and purity of the isolated lignins. Previous studies indicated that actions of ball milling continuously divide plant cell walls into finer particles. Thus, the released lignin by early ball milling may come from large domains of lignin in the cell wall, notably from the corner sections of the middle lamellae [84, 85]. Degradation methods have been applied to MWLs and the wood meal residues after solvent extraction [86]. Results from these studies suggested that MWLs resemble more to lignins located in the middle lamellae although lignins located in secondary cell walls can be extracted out by aqueous dioxane. However, studies from Maurer and Fengel [87] showed that the compound middle lamella is more resistant against ball milling than the secondary cell wall. Cell wall corner and middle lamella fragments remain even after a long period of ball milling, whereas the secondary wall is broken down quickly and cellulose in the secondary wall lose its crystalline as observed under polarized light. After aqueous dioxane extraction of ball-milled wood from spruce and beech, the removal of lignin from particles was seen with Pt/C-replica by electronic



**FIGURE 6.6** Coupling of two oligomers is the source of most of the 5-5 and 4-O-5 linkages. The 5-5 structure is produced only from two guaiacyl phenolic units; coupling with a monolignol  $M_G$  or  $M_S$  results in a dibenzodioxocin product. A  $\beta$ -O-4 unit may also couple at its 1 position with a monolignol  $M_G$  or  $M_S$  at its  $\beta$  position producing a spiro dienone structure.

microscopy and with densitometric evaluation of UV-micrographs. Results from this electronic microscopy and UV-micrographs studies indicated that MWLs are mainly from secondary cell walls. Recently, ball-milling effects on lignin structures have been studied by two groups of researchers. Ikeda et al. [88] analyzed lignins in wood, MWL, CEL, and residual lignin (REL) from a loblolly pine using a modified derivatization followed by reductive cleavage (DFRC) method and nitrobenzene oxidation. Results from this study indicated that vibratory ball milling does not change the lignin structures as long as some precautions are taken, i.e. dry ball milling under nitrogen atmosphere causes substantial structural changes

whereas ball milling in toluene has little effect on the lignin structures. Therefore, MWL and CEL are representative of the total lignin in wood although they have higher phenolic hydroxyl contents. Fugimoto et al. [89] applied ozonation analysis to ball-milled wood obtained under various ball-milling conditions to investigate milling effects on lignin structures. It was found that regardless of what conditions used in this study, ball milling does degrade lignin through  $\beta$ -ether cleavage and prefers *erythro* degradation. The degree of  $\beta$ -ether cleavage was estimated to be about 25% when ball milling released 40% of lignin. An interesting result from this study was that ball millings under aerobic and anaerobic conditions



**FIGURE 6.7** Cross coupling reactions between coniferyl alcohol and ethyl ferulate produce various cross coupled products including  $\beta O 4$ ,  $8 \beta$ ,  $\beta 5$ , and  $8 5$  cross coupled dimers. The carbon atom numbering follows arabic the numbering system for ferulate and mixed (Arabic and Greek) numbering system for coniferyl alcohol, as is mostly used in cell wall chemistry fields.

resulted in the same degree of degradation. Therefore, these results suggested that structural changes caused by ball milling are not preventable and that mild ball-milling conditions combined with enzymatic treatments should be used to isolate lignins, with less modification, in higher yields.

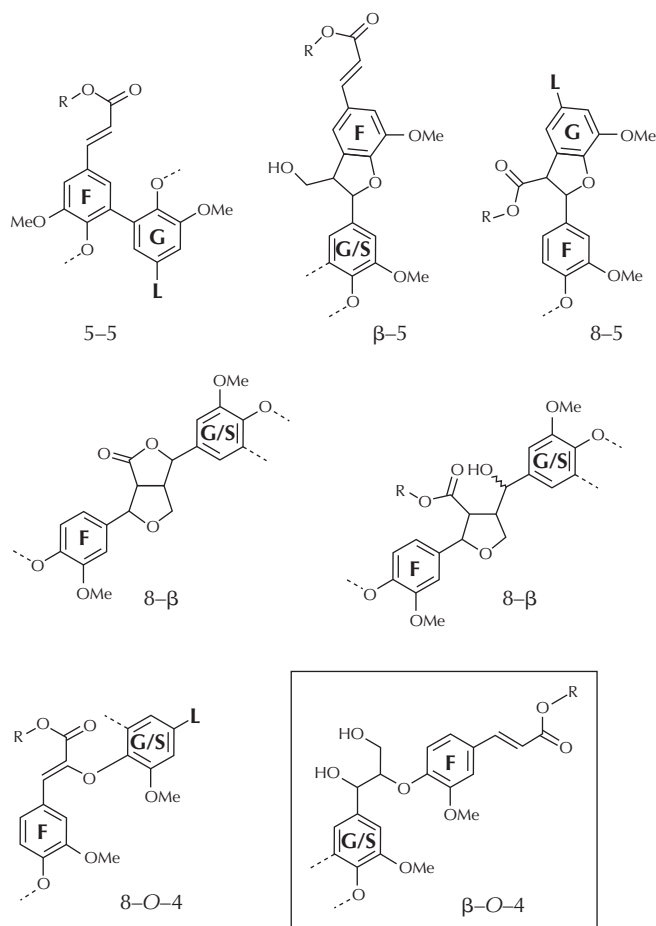
Although the MWL procedure was designed to isolate lignin from wood plants, many researchers have used this method with or without the modifications to obtain lignins from various grass plants as standard preparations to perform structural and compositional studies [74, 90–93]. Jung and Himmelsbach [94] developed a method for the isolation of lignin from wheat straw by ball milling and enzyme treatment. After examination of various variables (milling time, enzymatic treatment time, and compositions of solvents for extraction), they suggested that milling for 8 days followed by cellulose hydrolysis for 4 days are needed to maximize the isolated lignin yield. Extraction of ball-milled, enzyme-treated straw with 50% dioxane–water produced lignin preparations with the highest yield and the similar structures as the lignin extracted by the standard method (96% dioxane). However, lignin extracted with 96% dioxane from ball-milled wheat straw contains the

least carbohydrate contamination and the highest concentration of hydroxycinnamic acids.

### 6.3.2 Enzyme Lignin

Another improvement of lignin isolated from ball-milled wood has been made by Pew and Weyna [95, 96] with the use of cellulolytic enzymes. In this case, cellulolytic enzyme was used to remove most of the polysaccharides from ball-milled wood meal before aqueous dioxane extraction. The resultant preparation obtained as an insoluble residue called milled wood enzyme lignin (MWEL) contains almost all of the lignin and as much as 12% of the polysaccharides. Several years later, Chang et al. [77] isolated lignin by extracting MWEL with 96% aqueous dioxane to produce a lignin preparation called cellulolytic enzyme lignin (CEL). The procedure is summarized in Fig. 6.10 [80]. CEL was found to be structurally similar to MWL, but it was obtained in higher yield with less degradation, and hence it is more representative of the total lignin in wood. However, this procedure is tedious, and the enzyme treatment requires 10 days or more [76, 97],





R = Polysaccharides

**FIGURE 6.8** Potential structures from ferulate cross coupling bridging polysaccharides and lignin in grass cell walls. Ferulate may cross couple with monolignols producing crossed dimers (Fig. 6.7) that continue to couple with monolignols forming lignin with  $\beta$  5,  $\beta$  8, and  $\beta$  O 4 cross coupled structures. Ferulate may also cross couple with lignin oligomers, forming ferulate lignin complexes with 5 5, 8 5, and 8 O 4 cross coupled structures. Current solvolytic methods release cross coupled ferulate only from  $\beta$  O 4 coupled structures.

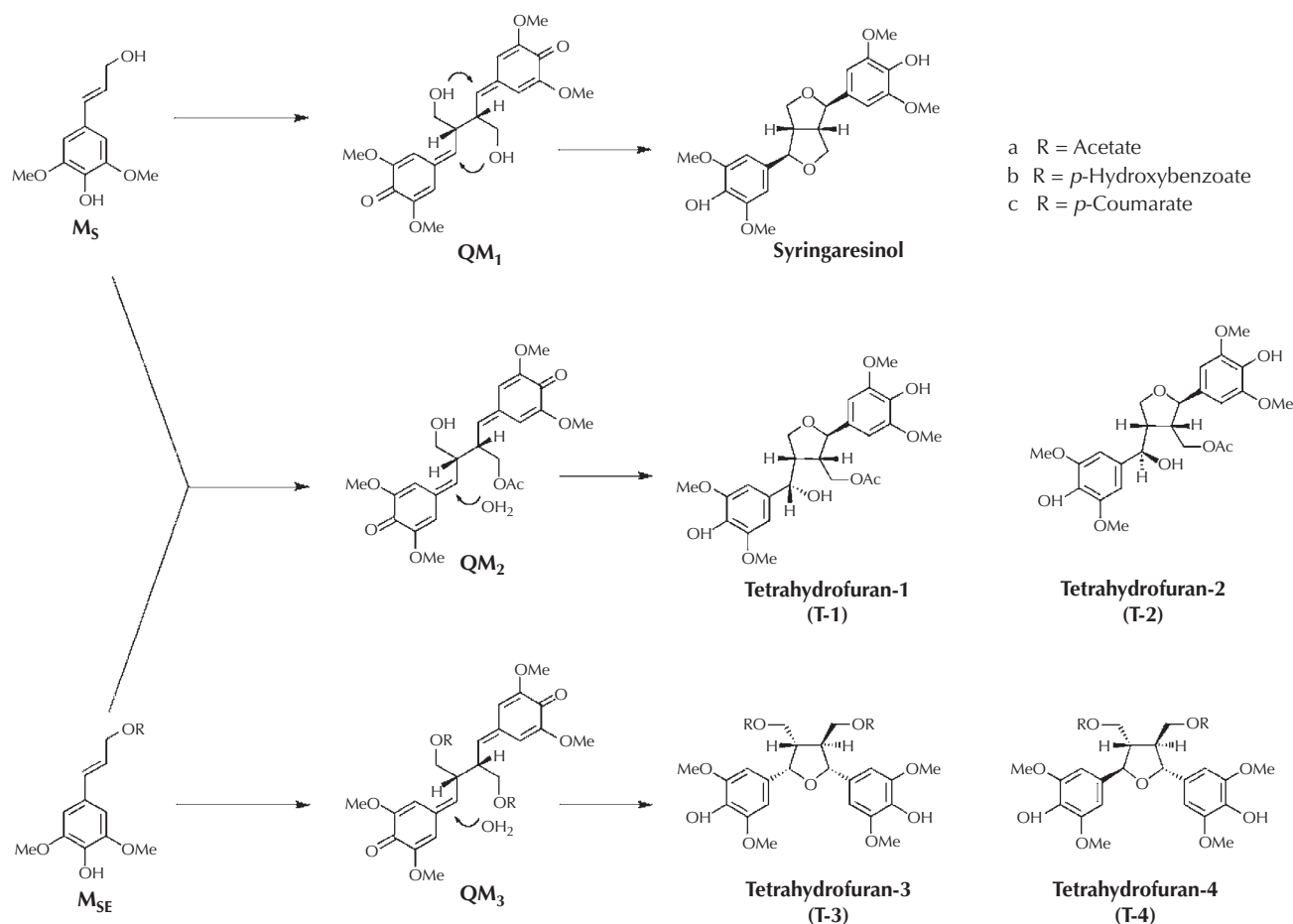
because the cellulose is highly crystalline and inaccessible, which impairs the physical contact between cellulose and enzyme. To cope with this problem, swelled enzyme lignin (SEL) method was developed [98]. Ball-milled wood was swelled in an organic solvent, then regenerated in water and treated with cellulase. Swelling opens up the cellulose crystalline or destroys them, loosens and reduces the physical associations between lignin and hemicelluloses. As a result, prior swelling should not only shorten the digestion time but also lead to a more intensive enzymatic degradation of the polysaccharides.

A novel procedure using the combination of enzymatic and mild acidolysis (enzymatic mild acidolysis lignin [EMAL]) was proposed by Wu and Argyropoulos [99] to isolate lignin that is more representative of the total lignin present in milled wood. The insoluble material remained

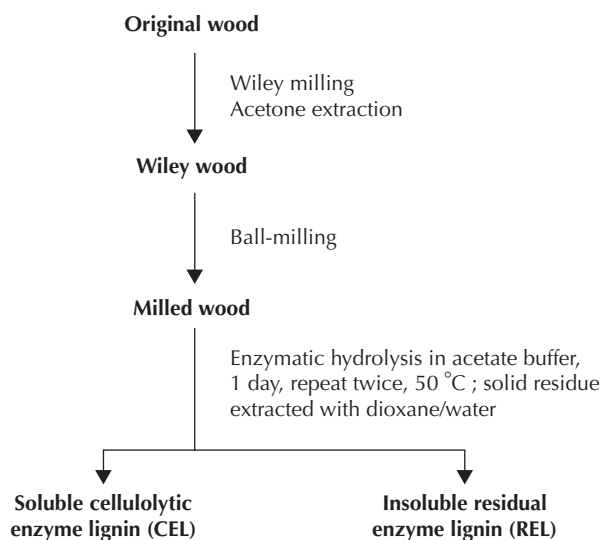
after the enzymatic hydrolysis was collected by centrifugation, washed twice with acidified deionized water (pH 2), and freeze-dried. The crude lignin obtained was further submitted to a mild acid hydrolysis using an azeotrope (bp, 86 °C) of aqueous dioxane (dioxane/water 85:15, v/v, containing 0.01 mol/L HCl) under an argon atmosphere. The resulting suspension was centrifuged, and the supernatant was carefully withdrawn, neutralized with sodium bicarbonate, and finally added dropwise to 1 l of acidified deionized water (pH 2). The precipitated lignin was allowed to equilibrate with the aqueous phase overnight, and it was then recovered by centrifugation, washed (two times) with deionized water, and freeze-dried. It has been reported that the yield of EMAL from Norway spruce is about four times greater than that of the corresponding MWL and about two times greater compared with that of CEL isolated from the same batch of milled wood [100]. More importantly, in comparison with MWL and CEL, no extent of structural changes has been verified in EMAL, and this indicates that EMAL is released by cleavage of lignin–hemicelluloses bonds rather than other linkages within the lignin macromolecule [101].

### 6.3.3 Alkali Lignins

Wood lignins require rather drastic hydrolytic treatments (5% sodium hydroxide [NaOH], 130–170 °C) to become soluble in aqueous alkali solutions; consequently such alkali lignins obtained by acidification of the hydrolysates are not particularly useful lignin preparations. In contrast, straw and grass lignins can be isolated in substantial yields by mild alkaline treatments, even at room temperature [75, 102, 103]. Therefore, lignins isolated through alkaline extraction of straw and grass samples have been recommended as particularly suited preparations for structural studies. Recent work has shown that about 60–70% of the lignin from wheat straw is extracted with dilute alkali at temperatures lower than 100 °C. In woody materials such as pine and birch, these percentages reach only about 20 or 30%, respectively. Such mild alkaline treatment does not cause much chemical modification beyond the saponification of ester bonds between *p*-CA and lignin or ferulic acid and polysaccharides. The considerable proportion of free phenolic groups present in grass lignins seems to play an important role in the solubility properties of Gramineae lignins [104]. In addition, saponification of hydroxycinnamates linked to lignins is also considered to play a role in the high extraction yields [91, 105–108]. Surprisingly, the alkali lignins have rarely been utilized for the characterization of straw and grass lignins [75]. The main reason for this is that the alkali lignins, isolated by traditional one step precipitation, contain much higher amounts of nonlignin materials such as polysaccharides, protein, and ash (mainly silica), with hemicelluloses being the predominant



**FIGURE 6.9** Cross coupling reactions of sinapyl alcohol  $M_S$  and acylated sinapyl alcohol  $M_{SE}$  produce novel tetrahydrofuran (T 1, T 2, T 3, and T 4) dimers, of which T 1, T 2, and T 4 have been identified in lignin preparations from several plants, including corn. (Adapted from Lu and Ralph [33]).



**FIGURE 6.10** Preparation of cellulase enzyme lignin (CEL) and residual enzyme lignin (REL) [80].

impurities. To overcome this difficulty, a rapid two-step precipitation method was proposed and it will be discussed in the following part.

Various types and concentrations of alkali, as well as extraction time and temperature conditions have been used for the extraction of wheat straw alkali lignins [109–115]. The results obtained showed that increase of the alkaline treatment temperature and alkaline concentration result in increasing yields of alkali-soluble lignins. No significant effect of treatment temperature and alkali concentration was found on the structural changes of obtained lignins, which contained roughly equal amounts of noncondensed guaiacyl and syringyl units with few *p*-hydroxyphenyl units. It was also found that NaOH and lithium hydroxide are more effective than potassium hydroxide in the release of alkali lignin from wheat straw. Compared with the Björkman method, the alkali extraction is simpler and has higher lignin yields.

As a selective bleaching reagent, alkali (NaOH) hydrogen peroxide has widely been used to bleach high

yield wood pulps. Generally, addition of hydrogen peroxide into alkali aqueous solution promotes the removal of lignin from pulp or even raw materials due to the action of the perhydroxyl anion, which is a strong nucleophile and reacts with chromophores in the lignin [116–118]. Lignin dissolved in alkali hydrogen peroxide solution has found no other practical use except for fuel burnt in pulping mill. Moreover, few studies have dealt with structures of the alkali hydrogen peroxide lignin although mechanism of alkali hydrogen peroxide bleaching has been intensively investigated [119–124].

Besides as bleaching reagent, alkali hydrogen peroxide has been used as a pretreatment process for improving the digestibility of agricultural residues as animal feedstock [125]. Recently, alkali hydrogen peroxide has been used as a pretreatment process for the conversion of lignocellulosic biomass feedstock into biofuel [120, 121, 126–129]. Gould [120] found that approximately half the lignin present in agricultural residues, such as wheat straw, could be solubilized when the residue was treated at 25 °C for 16 h with an alkaline solution of hydrogen peroxide. The delignification was most effective at pH 11.5. High-temperature treatment can increase the reactivity of  $\text{H}_2\text{O}_2$  toward phenolic structures [130]. The studies of Sun's group showed that about 80% of the lignin in wheat straw is removed by treatment with 2%  $\text{H}_2\text{O}_2$  at 50 °C for 24 h under alkaline conditions [115]. Under such mild conditions, it was found that alkaline hydrogen peroxide lignin from wheat straw has similar composition and structure as the alkaline lignin.

### 6.3.4 Organosolv Lignin

Delignification of wood using organic solvents (“organosolv pulping”) has been the subject of considerable research activity since the idea was introduced later last century and has generated increasing interest [131]. Organosolv lignins are usually recovered from spent organosolv pulping liquor by precipitation in water after evaporating organic solvents. The most used solvents for organosolv pulping were primary alcohols with low boiling point such as ethanol and methanol although other solvents, namely acetic and formic acids as well as high boiling point organic solvents, have also been used.

Acids or bases are commonly used as catalysts in organosolv pulping although “uncatalyzed” organosolv pulping can be performed at relatively higher temperature. Under pure organosolv pulping (without catalyst) conditions, delignification is actually promoted by acetic acid released from thermo hydrolysis of acetates in raw materials. The rate of delignification in alkaline systems is governed by the cleavage of  $\beta$ -ether bonds in lignin. Under acidic conditions,  $\alpha$ -ether cleavage occurs to a great extent, and the likelihood of  $\beta$ -ether cleavage increases in more

strongly acidic systems [132]. The beneficial effect of methanol in methanol pulping may be ascribed to either improved lignin solubility or its reduced tendency toward condensation, whereas in ethanol pulping, aqueous ethanol penetrates easily into the structure of wood resulting in uniform delignification. Ethanol also reduces the surface tension of the pulping liquor, which improves the diffusion of other pulping chemicals, for example, alkali.

In spite of having advantages over the traditional Kraft pulping process, organosolv pulping still does not find commercial applications although several pilot-scale organosolv pulping plants have been established in the past. However, organosolv pulping processes, as pretreatment/biorefinery techniques for cellulosic biofuel production, have attracted increasing interest all over the world because of the potential to produce clean fractions – pure cellulose suited for enzymatic hydrolysis, lignins having potential high-value applications, and hemicelluloses (or degraded polysaccharides) for biofuel productions. Therefore, increasing efforts have been made to fractionate lignocellulosic materials by using various organic solvents with catalysts/additives.

Table 6.1 presents yields of organosolv lignins extracted with different organosolv mixtures under various conditions, and ethanol–water (60/40, v/v) mixtures under various conditions [133]. Apparently, using volatile organic solvents instead of inorganic chemicals resulted in slow delignification at low temperature. This result was in good agreement with the study of Vázquez-Torres et al. [134, 135]. In the previous study [133], treatment of wheat straw with 0.5 M NaOH at 75 °C for 2 h released 52% lignin, which was approximately double the yield obtained from organosolv systems. No significant difference in yields were observed for each solvent mixture (Table 6.1) except for the extractions with ethanol–water (60/40, v/v) in 0.5 and 1.0 N  $\text{H}_2\text{SO}_4$  at 75 °C for 2 h, which produced much higher yields of lignin, 32.5 and 43.4%, respectively.

The main variables that affect the extent of delignification and cellulose degradation of organosolv pulps are as follows: extraction temperature, extraction time, solvent composition, and hydrogen ion concentration [136]. In the range of conditions studied by Sun et al previously [115], it appeared that for wheat straw an ethanol–water system at a concentration of 60/40, v/v in 1.0 N  $\text{H}_2\text{SO}_4$  at 75 °C for 2 h provided a high rate of both delignification and decomposition of hemicelluloses. The yield of organosolv pulp was 66.1% that contained 21.2% hemicelluloses and 12.1% lignin, respectively. As shown in Table 6.1, there was no significant difference in the organosolv lignin yields among the various organic solvents used, which account for 24–28% of the total amount of acidic chlorite lignin present in wheat straw. Increase of ethanol–water volume ratio from 40/60 to 70/30 resulted in an increase in lignin yield from 25 to 31%, while the reverse effects from 31 to 24%, appeared an increase of the volume ratio from 70/30 to

**TABLE 6.1** Yield (% Acidic Chlorite Lignin) of Organosolv Lignin Under Various Extraction Conditions

Organic sol- vents/water (60/40,v/v) in 0.02 N H <sub>2</sub> SO <sub>4</sub> at 75 °C for 2 h	Yield (%)	C <sub>2</sub> H <sub>5</sub> OH/H <sub>2</sub> O in 0.02 N H <sub>2</sub> SO <sub>4</sub> at 75 °C for 2 h at different volume ratios	Yield (%)	C <sub>2</sub> H <sub>5</sub> OH/H <sub>2</sub> O, 60/40 at 75 °C for 2 h in different H <sub>2</sub> SO <sub>4</sub> concentrations	Yield (%)	C <sub>2</sub> H <sub>5</sub> OH/H <sub>2</sub> O, 60/40 in 0.02 N H <sub>2</sub> SO <sub>4</sub> at 75 °C for various times (h)	Yield (%)
Ethanol/water	27.7	40/60	24.8	0	20.0	1.0	24.1
Methanol/water	24.2	50/50	24.9	0.01 N	19.9	1.5	26.9
1 Propanol/water	27.1	60/40	27.7	0.02 N	27.7	2.0	27.7
<i>n</i> Butanol/water	23.9	70/30	31.2	0.03 N	27.6	2.5	27.8
Dioxane/water	24.2	80/20	29.8	0.1 N	27.9	3.0	28.0
Acetone/water	26.4	90/10	24.1	0.5 N	32.5	5.0	28.3
				1.0 N	43.4	12.0	28.7

90/10. The isolated organosolv lignin fractions contain low amounts of polysaccharide sugars (4.5–5.3%) and have low average molecular weights (MWs) (1200–1650 g/mol). These results generally are comparable to the ball-milled lignin, enzyme lignin, and alkali lignin obtained from wheat straw.

During organosolv acid delignification, the Acetosolv process (based on the utilization of HCl-catalyzed acetic acid media) and Formacell process (formic acid-catalyzed media) have proved to be promising process to achieve total utilization of lignocellulosics without impact to environment. Both processes have ability to extensively remove both lignin and hemicelluloses under mild conditions, without significant cellulose degradation. By either of these processes [137–139], wood and nonwood materials can be fractionated to pulp, lignin, and low MW or degraded carbohydrate products, which makes it easy to utilize them as value-added products. In the previous study [140], treatment of wheat straw with acetic acid–H<sub>2</sub>O (65/35, v/v), acetic acid–H<sub>2</sub>O (80/20, v/v), acetic acid–H<sub>2</sub>O (90/10, v/v), formic acid–acetic acid–H<sub>2</sub>O (20/60/20, v/v/v), formic acid–acetic acid–H<sub>2</sub>O (30/60/10, v/v/v), using 0.1% HCl as a catalyst at 85 °C for 4 h, in which 78.2, 80.0, 88.2, 89.4, and 94.1% of the original lignin was released, respectively. The results showed that aqueous organic acid is more effective than aqueous organic alcohol as reagents for delignification and fractionation of cellulose, lignin, and hemicelluloses from the straw. In particular, formic acid has a significant effect on the dissolution of lignin. All the isolated organosolv lignins contained 0.9–4.3% of contaminated hemicelluloses and had weight-average MW between 3960–4340 g/mol.

High boiling solvents (HBS) such as ethylene glycol (b.p. 197 °C) [141, 142] and tetrahydrofurfuryl alcohol (b.p. 178 °C) [143] were used for pulping. Recently,

Kishimoto et al. [146, 147] showed that 1,3-butanediol (b.p. 208 °C) and 1,4-butanediol (b.p. 232 °C) are very effective delignification solvents for both hardwoods and softwoods even without acid catalyst at 200 and 220 °C, respectively. The solvents can be reused repeatedly after removing the dissolved lignin from waste liquors by precipitation with water, although they contain sugars, soluble lignin and their modified compounds. It was found that the recovered solvents were even more effective in pulping than fresh solvents. To investigate the delignification mechanism under HBS pulping conditions, a phenolic  $\beta$ -O-4 type lignin model compound, guaiacylglycerol- $\beta$ -guaiacyl ether was treated with 70% aqueous 1,3-butanediol solution at 160–200 °C and 70% aq 1,4-butanediol solution at 180 °C, respectively [146, 147]. It was found that most of the reaction products were generated by recombination of phenoxy radicals formed by homolysis of the  $\beta$ -aryl ether and the phenolic  $\beta$ -O-4 linkages in lignin were cleaved homolytically (radical mechanism) via quinone methide intermediates under HBS pulping conditions. MWL as well as wood meal from todo fir (*Abies sachalinensis* MAST) was also treated with 70% aqueous 1,4-butanediol solution at 220 °C without acid catalyst to elucidate the delignification mechanism during HBS pulping [148]. The decrease in average MW and the increase in phenolic hydroxyl groups in MWL indicate the depolymerization of lignin. And it was also found that the  $\beta$ -5 and  $\beta$ - $\beta$  substructures were relatively resistant to HBS pulping conditions [148, 149]. Interestingly, the addition of a reducing sugar to the fresh solvent accelerated the delignification rate dramatically [150, 151]. Further study of phenolic  $\beta$ -O-4 type lignin model compound in the presence of glucose revealed that addition of glucose increased the formation of guaiacol, coniferyl alcohol, and its  $\gamma$ -ethers, and decreased the formation of radical coupling compounds dramatically. These results suggested that



reducing sugars stabilize phenoxy radicals formed by homolysis of phenolic  $\beta$ -ethers. But  $\beta$ -aryl ether in nonphenolic structures was inert to HBS pulping.

### 6.3.5 Purification of Lignins

Many purification procedures have been attempted to reduce polysaccharide and other contaminants content in various isolated lignins. In this section, three main purification methods will be discussed briefly. The first purification method is the original one, which use two precipitations for the purpose of purification [78]. First, the crude lignin dissolved in 90% acetic acid was precipitated into water. The precipitation product is then further purified by dissolving in 1,2-dichloroethane–ethanol (2:1) mixture and by precipitating into ethyl ether. Some modified purification methods for crude lignin preparations have been reported. For instance [83], MWL is dissolved in pyridine–acetic acid–water (9:1:4, v/v/v), and the solution is extracted twice with chloroform. The organic layers from extractions are combined after centrifugation. The solvents are removed by evaporation (temperature  $<35^{\circ}\text{C}$ ). If complete removal of pyridine is desired, this can be achieved by repeated addition and removal of ethanol [152]. The residue is dissolved in 10–20 ml of 1,2-dichloroethane–ethanol (2:1, v/v), and the solution is added dropwise to magnetically stirred ether (250 ml). The precipitated lignin is centrifuged off, washed twice with ether, and dried in vacuum over  $\text{P}_2\text{O}_5$  and KOH. Purification according to this method provided products with a low carbohydrate content, namely 3.7% in birch lignin [153] and 0.2–0.3% in spruce lignin [154]. However, the loss of lignin during such intensive purification steps should be concerned if the representation of the lignin preparation is a priority.

The ability of solvents to dissolve or swell a variety of isolated lignins increases as the hydrogen-bonding capacities of the solvents increase and as their solubility parameters (after Hildebrand) approach a value of around 11. Lower MW lignin fractions are soluble in solvents with a wide range of solubility parameter and hydrogen bonding capacity than are the higher MW lignin fractions [155]. Further studies by Schuerch [155] also discovered that the solubility of lignin is better in the mixture than in either solvent alone. Therefore, various solvents or solvent mixtures can be used to fractionate lignins to produce fractions with high purity and narrow dispersity. In several studies on structural heterogeneity of kraft lignin, the lignin was subjected to fractionation before the characterization. Lindberg et al. [156] fractionated kraft lignin by successive extraction with organic solvents, and Lin and Detroit [157] by ultrafiltration of a kraft black liquor. Similarly, Mörck et al. [158, 159] fractionated kraft lignins derived from both softwood and hardwood, into five and three fractions with different MWs

by successive extraction with organic solvents. In the previous studies [160, 161], ash-AQ and soda-AQ lignins from the black liquor of oil palm empty bunch were fractionated into four fractions by successive extraction with dichloromethane (Fraction 1), n-propanol (Fraction 2), and methanol–dichloromethane (Fraction 3). The results showed that the lignin fractions 1–3 were free of bound polysaccharides, and the residue, after being extracted with organic solvents (Fraction 4), contained 1.26 and 2.78% associated hemicelluloses, respectively. It should be mentioned that in both cases the polysaccharides were significantly removed by the two-step purification method. Recently, the degraded *Eucalyptus pellita* kraft lignin in the black liquor of KP-AQ pulping was precipitated directly at  $\text{pH} \sim 2.0$  without further purifying to obtain lignin of more representative with a whole distribution of MW [162]. The precipitated lignin was fractionated into six fractions by successive extraction with organic solvents. It was found that all the degraded lignin fractions contained associated hemicelluloses. From Fraction 1 to Fraction 6, extracted with ethyl ether, methylene chloride, n-propanol, ethanol, methanol, and dioxane respectively, the hemicelluloses associated to the lignin fractions amounted to 0.24, 0.03, 6.3, 4.6, 5.5, and 0.9%, respectively.

Because of the low solubility of lignin in acidic aqueous media,  $\text{CO}_2$ ,  $\text{H}_3\text{PO}_4$ ,  $\text{H}_2\text{SO}_4$ , and HCl can be used to precipitate kraft lignin from black liquors.  $\text{CO}_2$ , which only brings the pH of the liquors to about 8–9, produces a rather low precipitation yield of pine kraft lignin, 60–80% [163, 164]. Lower pH values of the liquors and higher recovery of lignin (up to 95%) can be achieved with strong acids. Acid precipitated lignin from black liquors retains a substantial amount of mother liquor, including degraded carbohydrates and inorganic salts. So, the precipitated lignin usually needs a purification stage before it can be used [165]. To improve the precipitation and purification processes, many methods have been suggested [166]. Among them, a novel two-step precipitation method had been suggested by Sun et al. [167]. The concentrated black liquor was acidified to pH 6.0 by dropwise addition of 9.68 N  $\text{H}_3\text{PO}_4$  or 6 M HCl. The soluble polysaccharides were recovered by precipitation of the neutralized mixture with three volumes of ethanol. The lignin soluble in ethanol was then recovered by reprecipitation at pH 2.0, adjusted by the same acid, from the concentrated supernatant (ethanol) solutions. The precipitated lignin was washed with acidified water (pH 2.0) and air-dried. It must be pointed out that this novel two-step precipitation method can apply to all the isolated lignins aforementioned in this section. For instance, the content of neutral sugars and uronic acids in ball-milled wheat straw lignin and enzymatic lignin fractions obtained from 6-day ball-milled wheat straw and 3-day cellulose-treated residues were reduced to 2.86 and 2.36%, respectively, after purification with this two-step precipitation method. Purification of all alkali lignins isolated from various plants



by using this two-step precipitation method produced pure alkali lignin fractions with less than 2% of associated polysaccharides.

## 6.4 ANALYTICAL METHODS FOR LIGNIN CHARACTERIZATION

Analytical methods for lignin characterization play important roles in understanding compositions and structures of lignins. Because of the complexity of lignin, it always has been and will be a challenging to develop suitable tools for characterizing lignins in plant cell walls or in lignocellulosic materials following a wide variety of chemical, physical, and biological treatments. Many methods have been developed for lignin analysis, qualitatively and quantitatively, although there is still a need to develop new methods capable of solving problems associated with lignins [18].

Generally, analytical methods for lignin characterization are classified into two groups: destructive (degradative) methods and nondestructive methods. The first group includes acidolysis, thioacetolysis, thioacidolysis, nitrobenzene oxidation, hydrogenolysis, nucleus exchange method, permanganate oxidation, copper oxide oxidation, and ozonation. The second group consists of various microscopic and spectroscopic methods such as UV microscopy, interference microscopy, Fourier transform infrared (FTIR) spectroscopy, Raman spectroscopy, and NMR. Details about these methods have been described in several books and review articles.

It is not our intention to describe all available methods for lignin analysis in this section. Only thioacidolysis, the DFRC method, and 2D NMR spectrometry are discussed in light of their unique usefulness or popularity for characterizing lignins in grasses.

### 6.4.1 Thioacidolysis

Thioacidolysis was developed by Lapierre, as an extension of acidolysis, to cleave  $\beta$ -aryl ethers in lignins so that the basic units linked by  $\beta$ -aryl ethers are released as monomers to be quantified by gas chromatography (GC) [168–170]. Thus, the yields of monomers released by thioacidolysis reflect the proportion of “uncondensed” units in lignin [171]. It is performed in dioxane/ethanethiol with boron trifluoride etherate as acid catalyst at 100 °C for 4 h. By this treatment, lignins are degraded through  $\beta$ -ether cleavage into monomers, dimers, trimers, and oligomers (Fig. 6.11). Thioacidolysis monomers can be analyzed by GC. Following thioacidolysis, Raney nickel desulfurization allows GC analysis of dimers and trimers resulting from condensed structures in lignins [172–174].

When thioacidolysis is performed on premethylated lignin samples, the 4-*O*-methylated monomers derived from

$\beta$ -ether linked phenolic units provide information about uncondensed phenolic units, although complete methylation of lignin or lignocellulosic samples with diazomethane is time consuming [175, 176].

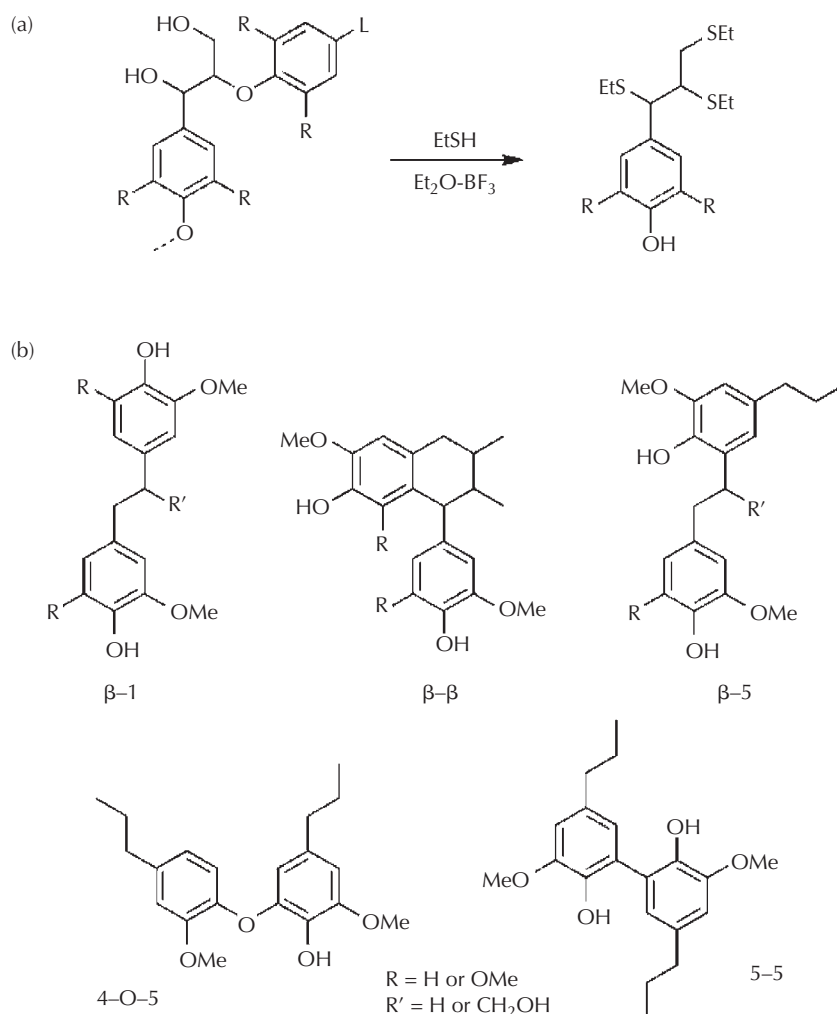
So far thioacidolysis is undoubtedly the most used analytical method for lignin analysis to obtain information about uncondensed structures (S/G/H ratios) and condensed structures (dimers compositions). However, when thioacidolysis is applied to grass lignins, esters on grass lignins may decrease the efficiency of  $\beta$ -ether cleavage by thioacidolysis [177, 178]. Therefore, one should be aware of this when interpreting thioacidolysis results for grasses. However, hydroxycinnamates in grass plants do produce diagnostic compounds after thioacidolysis [171].

Compared with acidolysis that had been used intensively before, thioacidolysis gives much higher yields of degradation monomers from uncondensed lignins [170]. Replacing ethanethiol with methanethiol gave somewhat higher monomer yields as reported by Önnérud [179]. When preswelled samples were used for thioacidolysis, monomer yields were apparently 25% higher with spruce wood than those obtained under standard conditions [173]. Thioacidolysis has been used for various lignin or lignin-containing samples including softwood, hardwood, and nonwood lignocellulosic samples.

### 6.4.2 DFRC Method

The DFRC method was developed by Lu and Ralph in 1997 as an alternative to thioacidolysis [180, 181]. Because of the mild conditions used and its unique selectivity, the DFRC method has become widely used for characterizing lignins from various origins [2, 80, 182–186]. It takes the advantage of the total dissolution of lignocellulosic materials by acetyl bromide (AcBr), and derivatizes  $\beta$ -aryl ether units in lignin producing bromo-ethers [187–189]. These bromo-ethers intermediates are efficiently cleaved by two-electron reduction, using zinc dust. Thus,  $\beta$ -ether linked units in lignin are released as hydroxycinnamyl acetates (Fig. 6.12). It is a unique feature of this method that it (partially) depolymerizes lignin back to its component monomers (although they are acetylated).

In addition to the main DFRC monomers (hydroxycinnamyl acetates) from  $\beta$ -ether linked guaiacyl/syringyl glycerol-1,3-diol units (major structures in lignin), other minor monomers from  $\beta$ -ether linked end-groups or units with  $\alpha$ -ketone oxidation states have also been identified [182, 190]. Dimers and trimers with  $\beta$ -5-,  $\beta$ - $\beta$ , 5-5-, and  $\beta$ -1-linkages were also isolated and identified from DFRC degradation products of softwood cell walls [191]. Two DFRC degradation products with isochroman structures isolated from the trimer fractions of loblolly pine wood degraded by the DFRC procedure implicated such isochroman structures in lignin, although they are simply a special



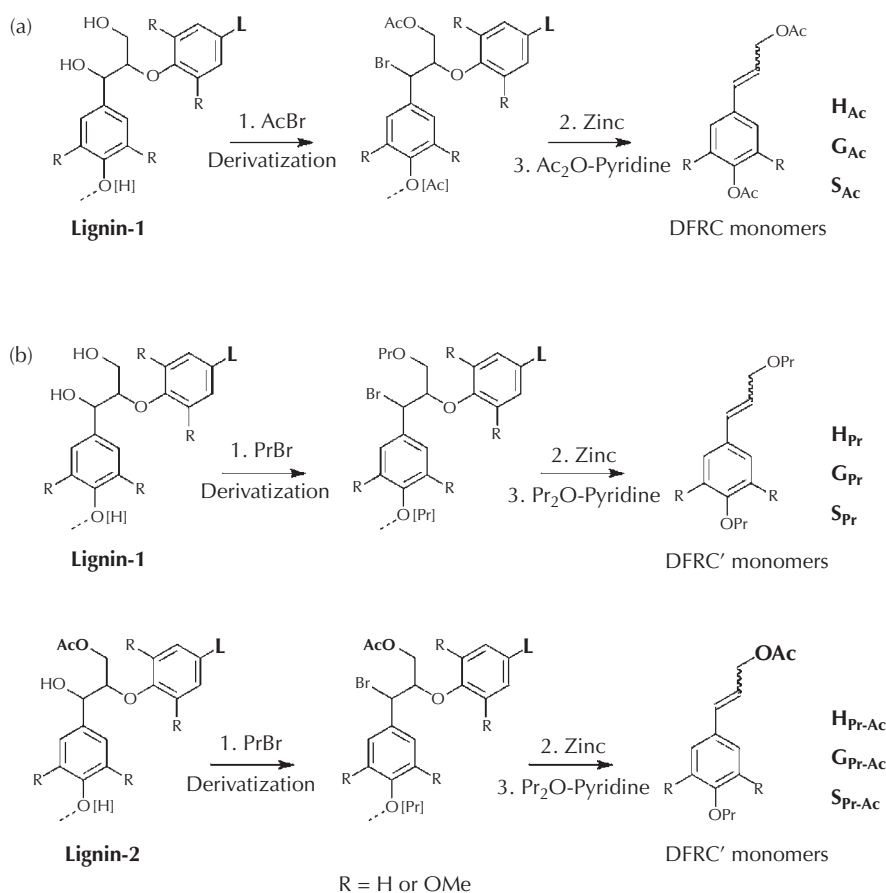
**FIGURE 6.11** (a) Thioacidolysis monomers recovered from the cleavage of  $\beta$  O 4 linkages in lignins. (b) Various dimers identified from thioacidolysis of lignins followed by a Raney nickel desulfurization.

case of  $\beta$ -1-units [192]. NMR analysis of the isolated MWL from the loblolly pine wood confirmed the existence of such structures. A plausible pathway leading to the isochroman structure during lignification was proposed [193].

One unique feature of the DFRC method is that esters on lignin remain fully intact during the DFRC procedure [194]. Thus, *p*-coumarates on lignins from grass plant materials can be detected and measured [57]. With a few modifications to the standard DFRC procedure by replacing the acetyl-containing reagents (acetic acid and AcBr) with the propionyl analogs, the modified DFRC (DFRC') method was applied to detect and quantify the naturally occurring acetates on lignins from some plants such as kenaf, aspen, or other nonwoody plants [195, 196] (Fig. 6.12). From these DFRC' degradation products, a series of  $\beta$ - $\beta$ -coupled degradation products with acetates on their  $\gamma$ -positions have been identified, which implicates novel tetrahydrofuran structures resulting from radical coupling of sinapyl acetate or cross-coupling between sinapyl alcohol and

sinapyl acetate in the kenaf lignin [197]. 2D NMR analysis of the kenaf lignin has confirmed the existence of such structures [32]. Analogs of such tetrahydrofuran structures have also been found in ester-containing lignins from various plants such as corn, palm, bamboo, and others [33, 198]. The existence of these tetrahydrofuran structures in naturally acylated lignins suggests that acylated lignins result from incorporation of acylated monolignols, through radical coupling reactions, during the processes of lignification. Acylated monolignols should, therefore, be considered as authentic lignin monomers.

The DFRC procedure, as a basic component, has been modified or combined with other techniques to provide more detailed and specific information about lignin structures [99, 101, 199, 200]. A recently modified DFRC method using deuterated reagents (acetic acid- $d_4$  and acetic anhydride- $d_6$ ) allows convenient differentiation of  $\beta$ -ether linked units with free phenols from those that are originally etherified, providing similar information about uncondensed



**FIGURE 6.12** (a) DFRC monomers H<sub>Ac</sub>, G<sub>Ac</sub>, and S<sub>Ac</sub> produced by the cleavage of  $\beta$  O 4 linkages via derivatization followed by reductive cleavage in lignin 1. (b) By replacing all acetyl reagents (acetyl bromide and acetic anhydride) with propionyl analogs (propionyl bromide and propionic anhydride), the modified DFRC (DFRC') method releases monomers H<sub>Pr</sub>, G<sub>Pr</sub>, and S<sub>Pr</sub> from normal lignins, lignin 1 whereas DFRC' monomers H<sub>Pr-Ac</sub>, G<sub>Pr-Ac</sub>, and S<sub>Pr-Ac</sub> from natural lignins having acetates on the  $\gamma$  positions of sidechain the naturally occurring acetates in lignin 2 survive DFRC'.

structures of lignin as that given by thioacidolysis of premethylated lignins [2].

### 6.4.3 Nuclear Magnetic Resonance Spectroscopy

Among the various physical and chemical methods for characterization of lignins, NMR spectroscopy has been shown to be reliable and comprehensive. In the early days, proton (<sup>1</sup>H) NMR was mainly used for lignin characterization. The first comprehensive review on NMR application for structural analysis of lignin was published in 1971 [201]. Detailed <sup>1</sup>H nuclear magnetic resonance (<sup>1</sup>H-NMR) data of lignin models and several isolated lignins were given in several reviews [201–203].

Semiquantitative measurement of some functional groups and qualitative analysis of structures in lignins have been made from <sup>1</sup>H-NMR spectra of acetylated or underivatized lignin preparations [204, 205]. However, because of the polymeric nature of lignin, diversity of protons from various structures, and irregularity of linkages

between building units in lignin, the <sup>1</sup>H-NMR spectrum of lignin is very broad and seemingly featureless.

With the development of NMR techniques and high-field NMR instruments, <sup>13</sup>C-NMR became popular for lignin structural analysis. There are several advantages of <sup>13</sup>C-NMR over <sup>1</sup>H-NMR. (1) Aided by the almost exact agreement between chemical shifts of carbons in good low-molecular-mass model compounds and in the polymer [206, 207], more detailed and defined information can be obtained from <sup>13</sup>C-NMR. (2) <sup>13</sup>C-NMR spectra are not complicated by spin–spin coupling so that only single signals are observed for each <sup>13</sup>C resonance when routine fully proton-decoupled <sup>13</sup>C-NMR spectra are recorded. (3) The <sup>13</sup>C-NMR has much wider range of chemical shift (about 240 ppm) than <sup>1</sup>H-NMR (about only 12 ppm) so that better resolution and less overlap of signals can be obtained in <sup>13</sup>C-NMR spectra of organic compounds, particularly of polymeric natural products, such as lignin. However, the extremely low abundance of the natural <sup>13</sup>C isotope makes <sup>13</sup>C-NMR much less sensitive so that long acquisition times are necessary to obtain satisfactory <sup>13</sup>C-NMR signals, especially for quantitative <sup>13</sup>C-NMR [202]. Also, despite

the spectral dispersion, considerable overlap of signals still occurs because of the incredible complexity of the lignin polymer.

Both solid-state and solution-state  $^{13}\text{C}$ -NMR have been used for structural characterization of lignins [208, 209], but solution-state NMR is more informative because of its better resolution, although soluble lignin preparations may not be representative of the whole lignin fraction in plants [210, 211]. The interpretations of  $^{13}\text{C}$ -NMR spectrum of lignin rely on NMR data recorded on synthesized low-molecular-mass model compounds. Many such models including monomers, dimers, trimers, and higher oligomers have been synthesized and characterized [212–216]. It was reported that NMR data (chemical shifts) taken from trimeric model compounds are better and more useful to help interpret  $^{13}\text{C}$ -NMR spectrum of lignin revealing more accurate structural features [62, 217].  $^{13}\text{C}$ -NMR spectra of synthetic lignins (dehydrogenative polymers [DHPs]) made from specific  $^{13}\text{C}$ -labeled monolignols revealed/confirmed identities of peaks from overlapped ranges in normal  $^{13}\text{C}$ -NMR spectra [218–220]. The selective  $^{13}\text{C}$ -enrichment of a specific carbon of protolignin in various plants has been achieved by feeding specifically  $^{13}\text{C}$ -enriched lignin precursors. By incorporation of such  $^{13}\text{C}$ -enriched lignin precursors into lignins into growing plants, more detailed structural features were revealed by solution-state  $^{13}\text{C}$ -NMR spectra of isolated lignins [221–224] and solid-state  $^{13}\text{C}$ -NMR spectra of whole plant cell walls [225, 226].

Although many distinct peaks can be assigned, there are many more overlapping peaks that cannot be identified [174]. With the development of modern NMR techniques, 2D NMR experiments, both homo- and heteronuclear, became popular and much powerful tools for lignin characterization providing richer and ambiguous information [227–232]. There is the potential to elucidate structures without first preparing model compounds, although such models are still crucial for most studies, and for obtaining additional evidence of the identity of new structural features in the polymer [232].

Among other 2D NMR experiments, the most used and useful are Heteronuclear Multiple-Quantum Coherence (spectroscopy) (HMQC) and HSQC experiments that provide correlations between directly bonded protons and carbons in two dimensions [233, 234]. Overlapping protons that are attached to carbons with different shifts are separated by their carbon shift differences, whereas overlapping carbons may be separated by their attachment to protons with differing chemical shifts. Therefore, the apparent resolution of 2D spectra is much better than anything that can be achieved in 1D spectra with today's field strengths. Therefore, quantitative measurement of various structures of lignins is possible when appropriate standards or pulse sequences are applied [234–236].

Figure 6.13 shows partial HSQC spectra of two lignin samples, in which C–H correlations of lignin sidechains from

the three major linkages,  $\beta$ -O-4,  $\beta$ -5 and  $\beta$ - $\beta$  are colored in blue, green, and purple. Aromatic C–H lignin unit correlations are shown in Fig. 6.14; it is evident that correlations from the 2-positions of guaiacyl units and those from the 2/6-positions of syringyl units are well resolved. Thus, it is possible that quantitative measurement of S/G ratios can be realized by integrating these correlations. Considering that it is possible now to have whole plant cell walls dissolved in NMR solvents, 2D HSQC NMR has great potential to be the method of choice for measuring S/G ratios in whole lignin and for profiling plant cell wall components [237, 238].

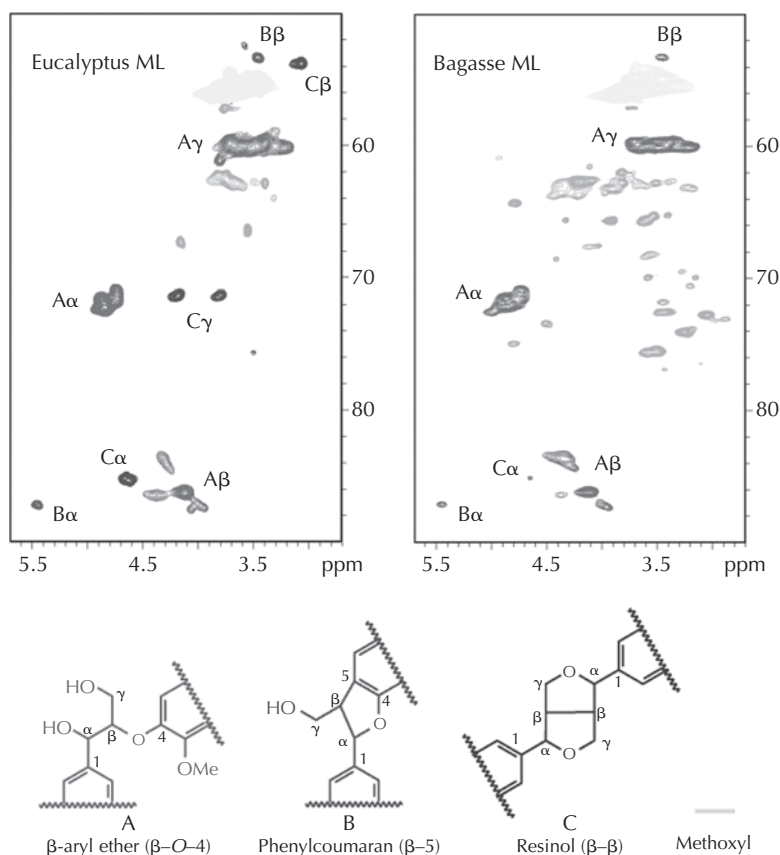
HSQC experiments have been used to characterize lignins in many circumstances [29, 30, 35, 233, 239–241]. Coupled with data from synthetic model compounds, HSQC NMR spectra of naturally acylated lignins demonstrated the existence of novel tetrahydrofuran structures in lignins, derived from radical (cross-)coupling of acylated sinapyl alcohol with sinapyl alcohols [32, 33, 242].

HSQC-TOCSY: An extension is the 2D HSQC experiment in which a Total Correlation Spectroscopy (TOCSY) transfer between protons is added prior to data acquisition. This relays the original proton–carbon correlation peak onto neighboring protons within the same spin-system, thus producing a  $^{13}\text{C}$ -dispersed TOCSY spectrum. This proves useful when analyzing complex proton spectra for which the 2D  $^1\text{H}$ – $^1\text{H}$  TOCSY spectrum becomes too crowded for unambiguous interpretation.

The assignments in HMQC/HSQC spectra (Fig. 6.13) must be made with good knowledge of both carbon and proton chemical shifts for each structural type. In contrast, in HMQC-TOCSY spectra (Fig. 6.15), knowledge of rough proton shifts allows very rapid identification of units because of the useful redundancy in the data. Knowing that acetylated  $\beta$ -ether units have  $\alpha$ -protons at  $\sim 6$  ppm,  $\beta$ -protons at  $\sim 5$  ppm, and  $\gamma$ -protons between 4 and 4.6 ppm, allows the blue contours to readily be assigned to  $\beta$ -ether structures A, for example. It is further easy to see which carbons are in the same structure – simply look for matching sets of correlations in the proton dimension.

Whereas the HMQC/HSQC-type spectra are valuable for their direct attachment information and because of the apparent extra dispersion they provide over 1D spectra, long-range  $^{13}\text{C}$ – $^1\text{H}$  correlation experiments Heteronuclear Multiple Bond Correlation (spectroscopy) (HMBC) provide enormously valuable connectivity data. Two- and three-bond C–H coupling constants are in the 2–15 Hz range, and HMBC [243] experiments are typically set with coupling evolution times of 60–120 ms, corresponding to coupling constants of 4–8 Hz.

HMBC NMR experiments have been applied to the characterization of lignins from various origins [92, 244–248]. This powerful tool provided unambiguous evidence that esters (*p*-coumarates, and acetates) are attached to the  $\gamma$ -positions of lignins and that most of these esters are on syringyl units, for example [74, 249].



**FIGURE 6.13** Partial HSQC spectra of milled lignins (ML) from Eucalyptus and bagasse showing the sidechain C–H correlations from the three main structural units (with  $\beta$ -O-4,  $\beta$ -5, and  $\beta$ - $\beta$  linkages) in lignins. (see color plate)

## 6.5 STRUCTURAL CHARACTERISTICS OF STRAW LIGNINS

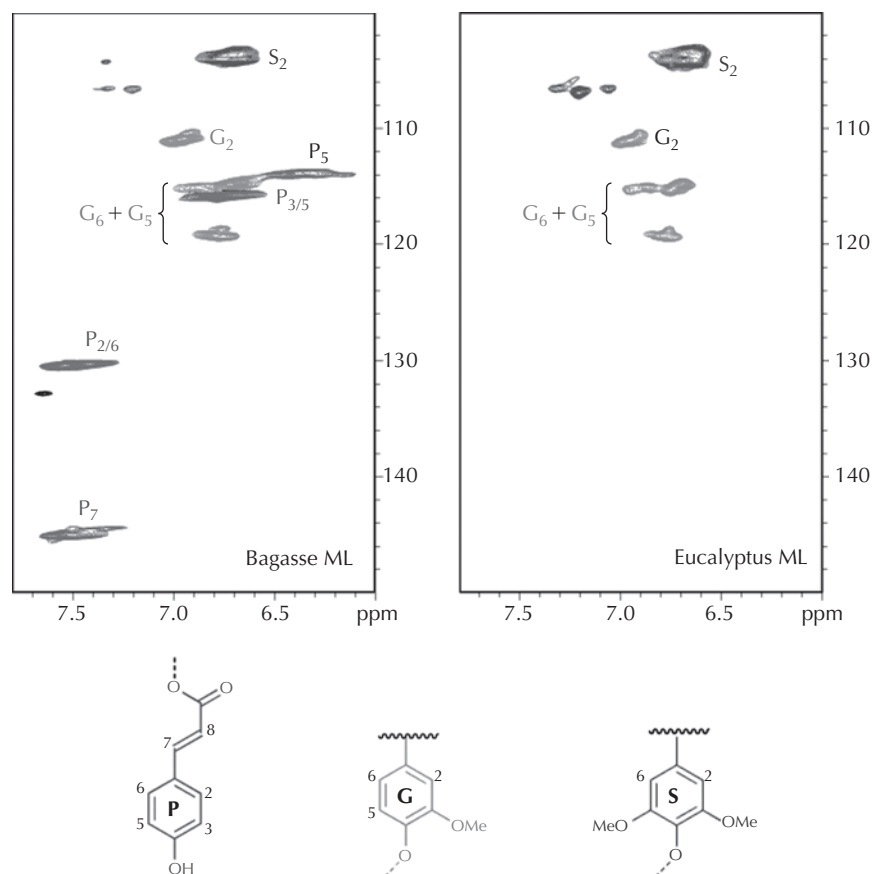
As one of major cell wall components, lignin consists of mainly three building units:  $P_H$ ,  $P_G$ , and  $P_S$  units, which are derived from  $M_H$ ,  $M_G$ , and  $M_S$ , respectively (Fig. 6.1). Various metabolic intermediates on the biosynthetic route to the monolignols, however, are also incorporated into lignins, particularly in mutant plants [34, 250]. The composition and structures of lignins varies considerably within and among plants. Thioacidolysis degradation products of lignins from various plants indicates that the proportions of  $P_H$ ,  $P_G$ , and  $P_S$  units in lignins are 4, 35, and 61% for mature maize stalks; 5, 49, 46% for wheat straw; and 15, 45, 40% for rice straw. In contrast to grasses, the lignins of dicot stems contain 14–66%  $P_G$  units, with the balance consisting of S units and only trace amounts of  $P_H$  units. In gymnosperms,  $P_G$  units comprise at least 95% of the lignin with the balance consisting of  $P_H$ . Although some gymnosperms do contain syringyl units, the trace amounts of  $P_S$  units often reported in “traditional softwoods” are questionable [36].

Lignins have been classified into three categories: gymnosperm (softwood), angiosperm (hardwood), and grass lignins. Later it was found that this classification is

inadequate because it leaves out most of the herbaceous angiosperm lignins, and some conifer families contain guaiacyl and syringyl types of lignins. Moreover, *p*-hydroxybenzaldehyde from nitrobenzene oxidation of grass lignins does not reflect the real contents of *p*-hydroxyphenylpropanoid units in lignins because *p*-hydroxycinnamate that is found mostly esterified onto  $\gamma$ -hydroxyl groups of lignin sidechains also contribute to the nitrobenzene oxidation product, *p*-hydroxybenzaldehyde. Thus, it was proposed by Gibbs that lignins should be classified into two major groups: guaiacyl lignins and guaiacyl-syringyl lignins according to their overall chemical constitutions instead of their taxonomy in biology. Nowadays, it is less often necessary to classify lignins based solely on nitrobenzene oxidation results because more advanced analytical methods are available.

Although straw lignins are classified as *p*-hydroxyphenyl/guaiacyl/syringyl (H–G–S) type lignins, the ratios among their nitrobenzene oxidation products do not present the true proportions of  $P_H$ ,  $P_G$ , and  $P_S$  units of straw lignins because of the overestimation of above-mentioned *p*-hydroxyphenyl unit – *p*-coumarates acylating lignin units that is not a part of the lignin “core” may produce *p*-hydroxybenzaldehyde on nitrobenzene oxidation. Whether *p*-CA-acylating lignin core in grass plant belongs





**FIGURE 6.14** Partial HSQC NMR spectra of milled lignins (ML) from Eucalyptus and bagasse showing the C H correlations from aromatic rings of structural units in lignins. The C H correlations from the 2 position of guaiacyl units and the 2/6 positions of syringyl units are well separated, and signals from *p* coumarates on bagasse ML are evident. (see color plate)

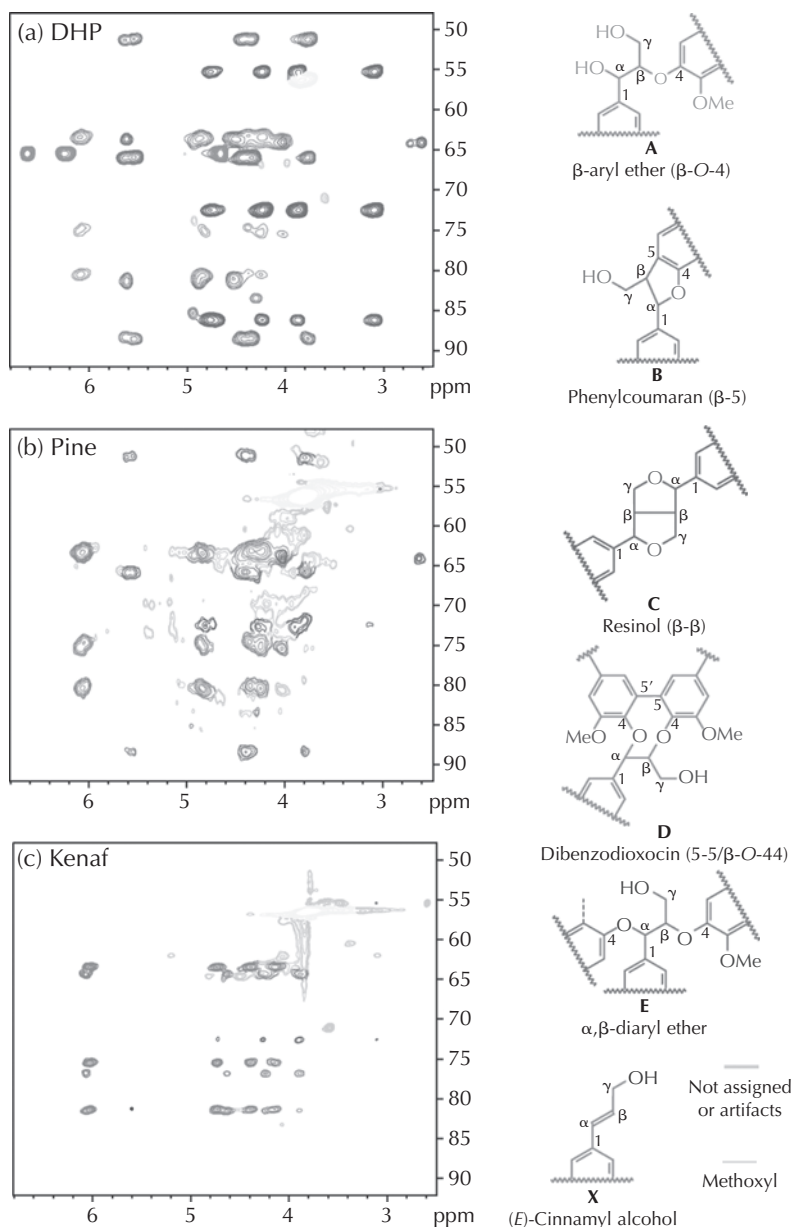
to lignin or not is still in debate but the *p*-coumarate contents should not be confused with derivation from the monomer, *p*-coumaryl alcohol. Unlike *p*-coumarates that simply adorn the lignin sidechains, ferulate (4-hydroxy-3-methoxycinnamate), acylating polysaccharide, is incorporated into grass lignins through free radical coupling mechanisms, forming variety of ether–carbon and carbon–carbon linkages [47, 243]. Therefore ferulates, and diferulates also derived from them by radical coupling, are now considered as an integrated part of grass lignins.

### 6.5.1 Ferulates in Grass Lignins

Ferulate acylates grass cell wall polysaccharides, notably to arabinoxylans at the C-5 position of  $\alpha$ -L-arabinofuranosyl moieties. During wall formation, feruloylated polysaccharides can be cross-linked by the radical coupling reactions of ferulate into dehydrodiferulates, dehydrotriferulates, or even higher oligomers [251]. Meanwhile, ferulate and diferulates may also copolymerize with monolignols such as coniferyl alcohol cross-linking carbohydrates, and lignin, i.e. producing so-called lignin–hydroxycinnamate–carbohydrate

complexes [68, 243, 252]. It has been long recognized that ferulic acid bridges carbohydrates and lignins through ester linkages onto carbohydrates and ether bonds onto the lignin moiety. However, how these bridges or cross-linkages are formed has been debated [47, 253]. Two distinct mechanisms have been proposed to be responsible for *p*-coumarate or ferulate etherification to lignin (Fig. 6.16).

Early on, a mechanism proposed for ferulate cross-linking to lignin was the simple nucleophilic addition of ferulate phenols to quinone methide intermediates generated from lignification [106] (Fig. 6.16a). It was described as a “passive” mechanism [254]. There is evidence for such reactions in vitro [255]. There are several reasons that this “passive” mechanism is not favored or not exclusive: First, ferulates are required to compete with other nucleophiles such as carboxylic acids, other phenolics, and water; Second, during lignification, water addition onto quinone methide intermediates forming  $\alpha$ -hydroxyl groups are dominant, whereas phenol addition onto quinone methide intermediates forming noncyclic  $\alpha$ -ethers is very minor if any occurs [47, 228]. However, one of the major proponents of  $\alpha$ -ethers reported that hydroxycinnamic acids (ferulic

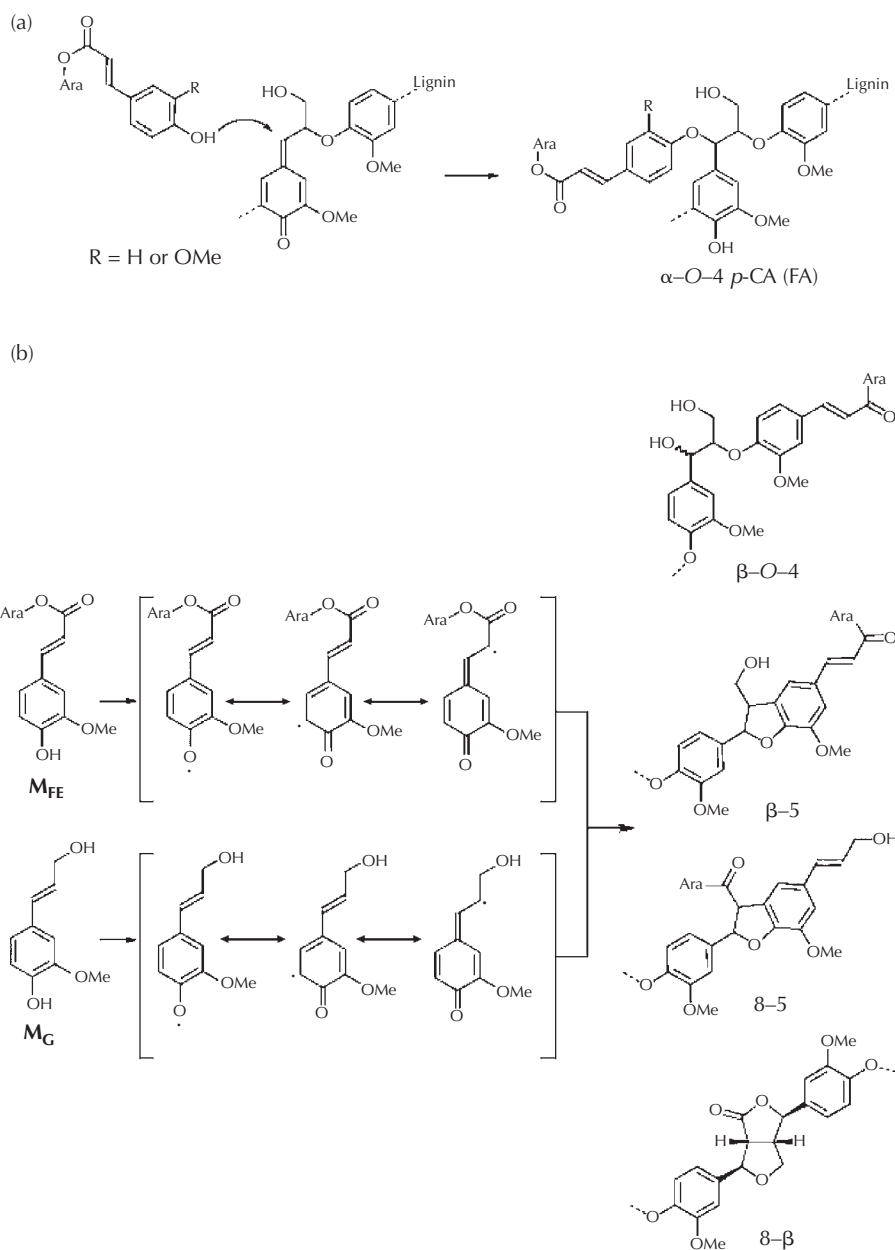


**FIGURE 6.15** HSQC TOCSY NMR spectra of a synthetic lignin (DHP) and two lignin preparations from Pine and Kenaf cell walls showing the C H correlations from sidechains of structural units with various linkage types in lignins. These C H correlations are aligned along each dimension according to their neighboring protons and protonated carbons (Adapted from Ralph et al. [232]). (see color plate)

acid and *p*-CA) are linked to lignin predominantly at the benzyl ( $\alpha$ -) position, not the  $\beta$ -position, in grass cell walls based on 2,3-Dichloro-5,6-dicyanobenzoquinone (DDQ) oxidation followed by base hydrolysis [253]. In this study, DDQ oxidation was utilized to oxidatively to release  $\alpha$ -ethers, so that DDQ treatment should help the release of more hydroxycinnamic acids than from untreated controls if there is any  $\alpha$ -ether linked hydroxycinnamic acids. However, the methods used have significant shortcomings. How DDQ oxidation affects  $\beta$ -ethers has not been tested. Our model studies show that  $\alpha$ -keto- $\beta$ -ethers (which are produced in the DDQ oxidation step from normal  $\beta$ -ethers) are cleaved under

room temperature base hydrolysis conditions if any oxidants (including peroxides in some ether solvents) are present. This has been reported in other contexts before [256]. Moreover, significant experimental errors could be generated if a small-differential number was sought by subtraction of two big numbers. This was exactly the case in the study mentioned earlier. The best way to resolve the  $\alpha$ - versus  $\beta$ -ethers dilemma is to develop an independent method that will allow an accurate quantification of  $\alpha$ - versus  $\beta$ -ether linked ferulates. Some approaches to solve these problems are undergoing evaluation.

Although the “passive” mechanism for ferulate cross-linking to lignin is not exclusive as we discuss next, ferulate



**FIGURE 6.16** (a) "Passive" mechanism proposed for etherification of hydroxycinnamates to lignins gives only one structure, the  $\alpha$  ether. (b) "Active" mechanism proposed for cross coupling of ferulates and lignins produces various structures from combinatorial radical coupling reactions.

$\alpha$ -ethers in lignin have been demonstrated in vitro and there has been indirect evidence for the existence of such linkages. Unfortunately, ferulates etherified to lignin via  $\gamma$ -positions of lignin sidechain have been proposed in the literature [23, 114, 257]. From the standpoint of chemistry or biochemistry, it is unlikely that these ferulate  $\gamma$ -ethers can be formed during lignification and no evidence has been found to support the existence of ferulate  $\gamma$ -ethers.

Finding ferulate–coniferyl alcohol dimers produced only from radical coupling reactions of ferulate and coniferyl alcohol would be a good indicator that ferulates in grass cell walls are capable of cross-linking carbohydrates and lignins

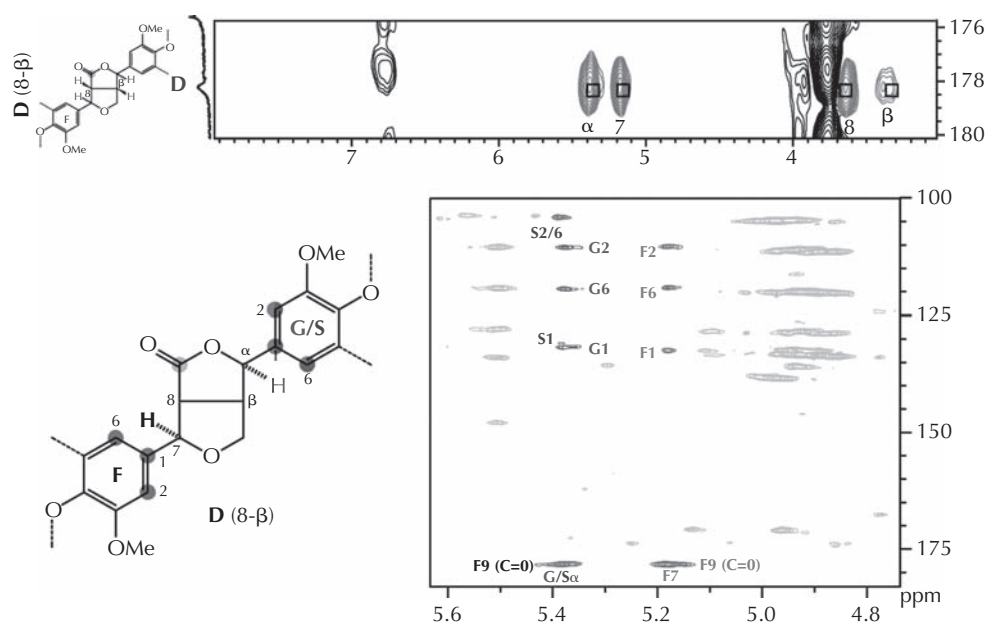
through free radical coupling mechanism. So far, a number of ferulate–coniferyl alcohol cross-coupled dimeric products including coniferyl alcohol– $\beta$ -O-4-ferulate, coniferyl alcohol– $\beta$ -8-ferulate dimers have been found in grass cell wall samples, although the presumably existing coniferyl alcohol– $\beta$ -5-ferulate and ferulate–8-5-coniferyl alcohol dimers had been missed. Now a new method has been developed for detection and measurement of these cross-coupled dimers [258]. By using this procedure, the target compounds – coniferyl alcohol– $\beta$ -5-ferulate and ferulate–8-5-coniferyl alcohol cross-coupled dimers have been detected in variety of grass cell walls and cereal-insoluble dietary

fibers. Identification of the range of cross-coupled dimers strengthens the contention that monolignols, at least coniferyl alcohol, readily react with ferulates via radical coupling reactions forming variety of cross-coupled products, and that such cross-coupled products may further react with incoming monolignols by radical coupling reactions as one part of the normal lignification process in grass cell wall development.

To further prove the “active” free radical mechanism, it is necessary to find unambiguous evidence that ferulates connect to lignins with variety of linkages that can be formed only via free radical mechanism. However, the relatively low level of ferulates in grass cell walls and the diversity of the possible structures resulting from the combinatorial free radical mechanism may prevent some powerful techniques, NMR for example, from readily detecting such structures in samples. Therefore, a first logical step was to determine if such structures could be generated and detected *in vitro* using simple ferulate esters. It was found in a model system that feruloyl carbohydrates react with monolignols and growing lignin polymers through free radical coupling reactions (active mechanism) forming synthetic lignins (so-called DHPs). Because of strategic [9- $^{13}\text{C}$ ]-labeling on ferulate to increase the sensitivity of NMR delineation, it was possible to observe a variety of structures resulting from various combinatorial coupling modes in these synthetic lignins [248]. It was also found that ferulates and diferulates in primary cell walls of maize readily react with coniferyl alcohol, and then incorporate into synthetic lignins produced within the cell

walls of suspension-cultured maize [252, 259]. In such a biomimetic system, nonlignified primary cell walls are lignified by feeding coniferyl alcohol and a source of hydrogen peroxide while using cell wall peroxidases to produce the radicals [260]. Studies with this biomimetic system showed that ferulate and 5-5-coupled diferulate have a greater propensity than 8-coupled diferulate to copolymerize with coniferyl alcohol, forming mostly 4-*O*- $\beta$ - and 8- $\beta$ - and some 8-*O*-4- and 8-5-cross-coupled products [259].

In addition to evidence from model studies, more compelling evidence came from the observation of 8- $\beta$ -cross-coupled ferulate–lignin structures in a ryegrass uniformly enriched with  $\sim 10\%$   $^{13}\text{C}$ , produced by growing the ryegrass in  $10\%$ - $^{13}\text{C}$ -enriched  $\text{CO}_2$  [243]. The major focus was on the 8- $\beta$ -coupled product that provided unambiguous evidence that ferulate–monolignol coupling was occurring *in vivo*. Indeed, the 8- $\beta$ -coupled product, a resinolide moiety, was compellingly ascertained by long-range 2D  $^{13}\text{C}$ – $^1\text{H}$  correlations experiments establishing that the carbonyl carbon was within three bonds of both  $\beta$ - and both 8-protons, all with NMR chemical shifts consistent with the model compound data (Fig. 6.17). Such a structure, the 8- $\beta$ -coupled resinolide product that can be produced only from radical 8- $\beta$ -cross-coupling of ferulate and a monolignol, implicates an internal transesterification reaction. Such a transesterification reaction has been demonstrated in a model system although we were always dubious of this reaction occurring in planta [65]. Along with such the 8- $\beta$ -coupled resinolide product, other cross-coupling products resulting from  $\beta$ -*O*-4-,  $\beta$ -5-/8-5-, 8- $\beta$ -cross-



**FIGURE 6.17** HMBC NMR spectrum of a Ryegrass milled lignin that has been  $10\%$   $^{13}\text{C}$  enriched. The required correlations for identifying the 8  $\beta$  structures of interest, D, are evident in the spectrum. The involvement of guaiacyl and syringyl units in such structures is also verified by the HMBC correlations. Synthesis of valid model compounds is absolutely crucial for the identification of new structures in complex natural polymer such as lignins. (Adapted from Ralph et al. [232]). (see color plate)

coupling reactions have been identified in such a model system, suggesting that there may be more of the possible combinatorial cross-products to be discovered.

Isolating representative lignins from plant cell walls has been and still is a challenge task for lignin structure research. It becomes more difficult to isolate grass lignins that are covalently bonded into other cell wall components. Because of cross-linking between carbohydrates and lignin by ferulates, lignins isolated by traditional dioxane–water extraction of ball-milled cell walls would have less cross-linking than those left in the residues after extraction. For nearly a century, it has been known that a substantial fraction of grass lignins (25–50%) can be solubilized by alkali at room temperature [261]. The alkaline solubility of grass lignins may be accounted for by two structural properties of these polymers. The first is the occurrence of the aforementioned ferulate cross-links between cell-wall polymers. The second and more likely reason is the higher frequency of free phenolic groups in grass lignins (and consequently lower MW), as compared with lignins in other types of plants. Therefore, a mild base treatment to hydrolyze esters between ferulic acid and polysaccharides would help to release lignins containing ferulic acid with ether linkages [91, 112]. Recently, two alkali lignin samples of corn and bagasse were isolated by mild base treatment (1 N NaOH, room temperature) of dioxane–water extracted ball-milled cell walls. Ferulate–lignin cross-linkages produced from  $\beta$ -5- and  $\beta$ -O-4-cross-coupling modes have been observed and identified in these two alkali lignin samples by 2D NMR experiments (Lu, unpublished). Finding such structures in lignin isolates provides further evidence that ferulate–lignin cross-linking is achieved through free radical coupling reactions.

The relatively ready identification of ferulate–monolignol crossed dimers that could be isolated from grass cell walls and model systems implied that ferulates were involved in cross-coupling reactions with monolignols. Although it is difficult to be certain to what extent ferulates also cross-couple with the growing lignin oligomers/polymers, the finding of such cross-coupled dimers implies that ferulates are at least partially involved with the very initiation of lignification – coupling of two monomers only occurs at the outset of lignin chain formation, the major polymerization reaction being the cross-coupling of a monomer with the free-phenolic end of a growing oligomer/polymer. This observation, therefore, leads to the hypothesis that ferulates (on polysaccharides in grasses) may act as lignin initiation sites, or nucleation sites (a term we favored to portray the idea of growth from a single element in a manner analogous to the way crystallization can nucleate from scratches in glass, for example) [243]. With the documentation of ferulates on polysaccharides in early cell walls in softwoods as well [262], the possibility exists that lignin initiation/nucleation by ferulates is a more general phenomenon.

## 6.5.2 *p*-Coumarates in Grass Lignins

Studies on lignin have showed that lignins in some plants are acylated by various acids: acetates in all hardwoods but at high levels in palms, kenaf, abaca, and sisal [32, 195, 196, 249]; *p*-hydroxybenzoates in palms, and *Populus* species (willow, aspen, poplar) [33, 263–268]; and *p*-coumarates in all grasses [74, 269–271]. *p*-Coumarate in grass lignins is mainly esterified to the  $\gamma$ -position of phenylpropanoid sidechains of S units in lignin as demonstrated by NMR studies with corn and wheat straw [74, 93]. Although some *p*-coumarates are esterified to arabinoxylans in immature tissues, most *p*-coumarate accretion occurs in tandem with lignification [272, 273], making *p*-coumarate accumulation a convenient indicator of lignin deposition in grasses.

There are several pathways possible for introducing such acylation. However it has now been rather unequivocally demonstrated for acetates in kenaf that acylation occurs at the monolignol stage [32]. The acylation by other acids (*p*-hydroxybenzoate, *p*-coumarate) logically follow a similar pathway. Thus, sinapyl *p*-coumarate in grass cell walls is then incorporated into the lignin polymer by polymerization and copolymerization with the traditional monolignols. Acylated monolignols implicate transferase enzymes in their synthesis. Researchers seem to be getting close to obtaining the enzymes involved and their genes. Monolignol *p*-coumaroylation in maize is via *p*-coumaroyl-CoA [274].

Acetylated lignin units may consist of up to 60% in kenaf lignin and *p*-coumarate content may reach up to 18% in corn lignin. It is quite evident that these monolignol conjugates can comprise a very high portion of the monomer pool for those lignins. Therefore, these acylated monolignols should be considered as lignin precursors in those plants in addition to the three monolignols (Fig. 6.2).

Acylation is, generally, heavy on syringyl units and little guaiacyl acetylation is detected [57, 177, 275, 276]. The acylation of the monolignol  $\gamma$ -OH does not greatly affect the course of the radical coupling reactions, but does markedly influence postcoupling rearomatization reactions of the resulting quinone methide intermediates QM (Fig. 6.9) [277] particularly in the cases where the  $\gamma$ -OH would normally be involved in that rearomatization (via intramolecular nucleophilic trapping reactions). For example, although homo-dehydrodimerization reactions are usually greatly favored over cross-coupling reactions (due to simple chemical compatibility), in vitro peroxidase-H<sub>2</sub>O<sub>2</sub> initiated coupling of equimolar sinapyl alcohol (M<sub>S</sub>) and sinapyl acetate M<sub>SE</sub> (R = acetate) produces an essentially statistical distribution (1:2:1) of the  $\beta$ - $\beta$ -dimers syringaresinol (S- $\beta$ - $\beta$ -S, from sinapyl alcohol + sinapyl alcohol), tetrahydrofurans T-1a and T-2a (from sinapyl alcohol + sinapyl acetate), and tetrahydrofurans T-3a and T-4a (from sinapyl acetate + sinapyl acetate) (Fig. 6.9) [32].



One initially surprising feature of *p*-coumarates on grass lignins is that they are simply terminal pendant groups. Their free-phenolic nature is readily evident from  $^{13}\text{C}$ -NMR spectra [74]. Despite their phenolic nature, *p*-coumarate esters on lignin units form few, if any, cross-linked structures mediated by radical coupling reactions of the *p*-coumarate moiety. On their own, in vitro or under conditions where radical generation capability is not limiting, *p*-coumarates will undergo radical coupling. But, there is no evidence that they do so in grass cell walls. The reason for this is that although *p*-coumarates interact with peroxidase to generate radicals, they quickly undergo radical transfer reactions with other phenolics, particularly sinapyl alcohol and syringyl units, producing more stable radicals (Fig. 6.18) [47, 277].

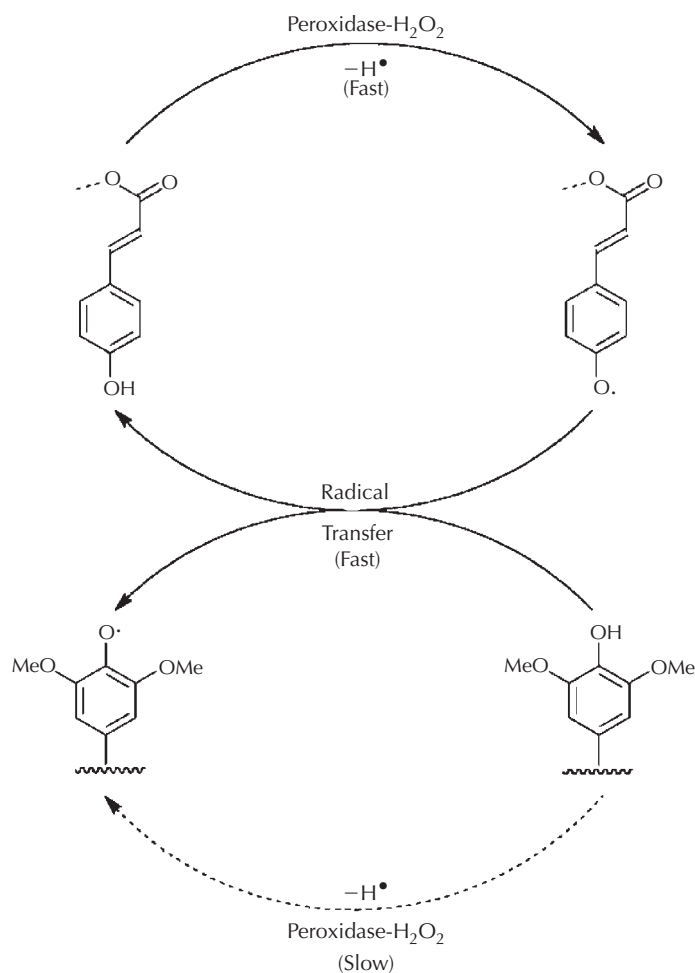
Whether this facilitation of sinapyl alcohol oxidation is a primary role for *p*-coumarates in grasses (and analogously, *p*-hydroxybenzoates in aspen, poplar, willow, and palms) is unknown. Although the above-mentioned in vitro studies indicate that *p*-coumarate esters enhance the oxidation of sinapyl alcohol [279–281], the artificial polymerization of

syringyl-rich lignins into primary maize walls was at times depressed by sinapyl *p*-coumarate because of accelerated inactivation of peroxidase and disruption of ferulate–lignin cross-linking [282].

Regardless, the *p*-coumarate radical's preference to undergo radical transfer to another phenolic present during lignification in grasses, over its radical coupling propensity, results in *p*-coumarates that are indeed part of the lignin polymer, but are not integrally incorporated into the polymer chains via radical coupling reactions – at least in the typical syringyl-guaiacyl lignins in grasses, they remain simply as free-phenolic pendant groups acylating the primary  $\gamma$ -OH of the lignin sidechain, albeit on a variety of lignin structures.

### 6.5.3 Lignin Carbohydrate Complexes in Grasses

Lignified plant cell walls are developed by successive deposition of cellulose, hemicelluloses, and lignins to form



**FIGURE 6.18** A diagram illustrating the possible mechanism by which *p* coumarate acts as an oxidative shuttle to enhance the oxidation of sinapyl alcohol during lignification of grass cell walls (Adapted from Hatfield et al. [277]).

a composite in which these components are physically and chemically connected to each other. Thus, lignins are always associated with other components especially with carbohydrates (hemicelluloses). The term “lignin–carbohydrates complex” (LCC) was first used by Björkman to describe a preparation of hemicelluloses accompanied by lignin [283]. Topics on LCC have been reviewed many times [15, 284, 285]. Evidence reported so far has strongly supported the existence of covalent bonds between lignin and carbohydrates. Four types of linkages between lignin and carbohydrates have been proposed: (1) glycosidic linkages; (2) ester linkages; (3) benzyl ether linkages; and (4) hemiacetal or acetal linkages. Among these linkages, the possibilities of glycosidic, benzyl ether, and ester linkages have been demonstrated with model compounds [286]. However, it has been extremely difficult to obtain unambiguous evidence on the nature and frequency of such linkages in real plants. The association of lignin and carbohydrates in grass cell walls is largely through free radical coupling of ferulates or diferulates with monolignols or growing lignin oligomers, although the other possibilities mentioned earlier cannot be ruled out.

LCCs have been studied intensively regarding their compositions and the nature of linkages between lignin and carbohydrates. Because of the complexity of LCCs, the isolation of homogeneous preparations is the most important step for the following compositional analysis and structural characterization. LCCs have been isolated by solvent extraction from various plant cell wall preparations; such as, (1) finely divided plant meal [87, 287–292]; (2) MWL [153, 154]; (3) residues remaining after enzyme and chemical treatment of the plant cell walls [293]; (4) chemical delignification liquors [294, 295]; (5) alkaline extracts from wood [296, 297]; (6) pulps [298, 299]; (7) derivatized wood [300, 301]; (8) synthetic lignin (DHPs) [302]; and (9) tissue culture [303, 304]. Among these materials, the finely divided plant meal and MWL were preferred sources for LCC isolation because any chemical or biochemical treatment before LCC isolation would break possible linkages between lignin and carbohydrates. Many isolation procedures have been proposed to obtain LCC preparations [286, 305, 306].

The LCC from herbaceous plants have been extracted with different procedures from various plant species. The most common and effective methods for LCC isolations were hot water and alkaline extractions of ball-milled cell walls. Water-soluble LCCs were prepared with hot water extraction followed by precipitation in ethanol [307]. These LCCs can be fractionated with size-exclusion chromatography to produce LCC fractions with different molecular sizes. It was found that extraction of ball-milled grass cell wall with dimethylsulfoxide (DMSO) and 1 N NaOH solution gives the highest yields of soluble materials [288]. Various LCC preparations from rice straw: bald cypress, and birch were obtained by 80% aqueous dioxane extraction

of ball-milled cell wall samples followed by a series of purification and fractionation steps [305]. Oxalic acid hydrolysis was used to disrupt arabinofuranosyl linkages releasing phenolic acid esterified only to arabinoxylans before DMSO extraction of LCC from ryegrass and barley straw [257, 308]. Examinations of these LCC preparations with methylation analysis, cellulase treatment and alkaline hydrolysis, and NMR indicated that ester and ether linkages between carbohydrates and lignin may exist in these samples. Results of thioacidolysis suggested that the DMSO-soluble fractions contain lignins with more condensed structures. Other types of alkaline-stable linkages such as benzyl ethers and glycosides, may also exist in the grass samples because a portion of the lignin remains firmly associated with carbohydrates after base hydrolysis [308].

Synthetic lignin (DHP)–carbohydrate complexes made from peroxidase-catalyzed polymerization of coniferyl alcohol in the presence of polysaccharides have been investigated to understand what types of linkages are possible between lignin and carbohydrates [309–311]. Analysis of these artificial LCC preparations implied that benzyl ether or ester and glycosidic linkages exist between lignin and carbohydrates.

Although the unambiguous evidence on the nature of direct linkages between lignin and carbohydrates in wood cell walls, if they exist, is still emerging, the cross-linking of lignin and carbohydrates via ferulates/diferulates in grass cell walls has been demonstrated as discussed in this chapter. It is generally accepted now that the cross-linking of carbohydrates and lignin by ferulates presents the greatest barrier to efficient grass cell wall utilization [26, 47, 261].

## 6.6 UTILIZATION OF LIGNINS

As one of the major components in plant cell walls, lignin plays very important roles in providing strength, rigidity, and resistance to pathogens [16, 312]. However, lignin has been always considered unwelcome in bioprocessing industries. Enormous quantities of lignin derivatives (technical lignins) are produced as byproducts from pulping processes that produce fibers from woody materials. It has been and is still a challenging task to use these lignins efficiently and cost effectively [313].

The history of lignin utilization traces back to the late 19th century when lignosulfonates produced from the bisulfite pulping process were claimed to be effective leather-tanning agents and dye-bath additives [313]. Since then, enormous effort has been dedicated to finding new uses for lignosulfonates. Nowadays Kraft pulping process is dominant for chemical pulp productions, but the majority of the kraft lignin produced from this process is burnt as fuel to drive the process and to recover valuable inorganic pulping chemicals. The major challenges for wide utilization of

**TABLE 6.2 Applications of Lignin Products**

Applications	Performance property
Carbon black and pigment dispersion	
Cement and concrete	
Dyestuff formulations	
Expander in lead and acid batteries	Dispersing
Gypsum wallboard	
Wettable and flowable pesticides	
Oil well drilling	
Boiler and cooling water treatment	
Corrosion inhibition	
Industrial cleaner	Complexing/dispersing
Micronutrients	
Protein precipitation	
Adhesives for board and veneer	
Animal and poultry feed pellets	
Ceramic and refractories	Binding
Foundry sands	
Ore and coal briquettes	
Soil conditioning	
Asphalt emulsion	Emulsion stabilizing
Wax stabilizer	

*Adapted from Lin [313].*

technical lignins relate to the inherent complex nature and the lack of good understanding or information about their chemical or physical properties [12].

Major utilization of lignins is based on their ability to function as dispersants, binders, emulsifiers, and sequestrants (Table 6.2). We do not intend to review all aspects of lignin utilization. Focus here is on some developments on the use of lignins as polymeric materials, biochemicals and biofuels.

### 6.6.1 Wood Adhesives Made from Lignins

Adhesives currently used for wood-panel products are all formaldehyde-containing resins including phenol–formaldehyde (PF), urea–formaldehyde (UF), melamine–

formaldehyde (MF), and resorcinol–formaldehyde (RF), although new formaldehyde-free adhesives are emerging as alternatives for wood composite production [314–316].

Although lignin present in plants acts together with hemicelluloses as a perfect natural adhesive for cellulose fibers, isolated technical lignins generally are poor binders for wood composites compared with conventional resin systems such as PF resin [317]. Because of the phenolic nature of lignin polymer, there have been attempts to replace phenol with lignin derivatives in PF resins to formulate wood composite adhesives suitable for plywood, particleboard, and waferboard. Produced as byproducts from sodium-, calcium-, and ammonium-based sulfite spent liquors (SSL), lignosulfonates were the most used technical lignins for making lignin-based adhesives. Such lignin derivatives in SSL have been used directly for formulating wood adhesives, but in most cases they were isolated, purified, and modified before used for producing adhesives [318].

Adhesive made directly from crude calcium-based SSL has been evaluated on a mill scale, but it was not commercialized because of the required high pressure and temperature for resin curing as well as the frequency of fires experienced during mill-scale trials.

Technical lignins purified by ultrafiltration process were better materials for making lignin-based PF adhesive [319]. Adhesives made from ultrafiltration-fractionated ammonium-based SSL were examined with waferboard by Shen and Calve [320]. The best board properties were obtained with low-MW (<5000) lignosulfonates whereas fractions with high MW (>5000) gave boards with poor mechanical properties. However, Forss and Fuhrman claimed that adhesives made with phenolic resin and high-MW kraft lignin fractions show improved adhesive strength [319].

Another approach to improving the strength properties of lignin-based adhesives is to increase the reactivity of technical lignins toward formaldehyde. Modifications including demethylation [321], hydroxyalkylation [322], and phenolation [323] have been used to improve the reactivity of lignins. Gupta and Sehgal showed that demethylation imparts better reactivity to kraft lignin and better glue adhesion properties are obtained with resin made from the demethylated lignins [324]. Hydroxymethylation (methylation) introduces hydroxymethyl group mainly onto C-5 position of phenolic guaiacyl units in lignins when lignins are treated with formaldehyde under base conditions. This reaction was used to increase cross-linking sites for reaction with PF resin, but studies by Chen and Gratzl showed that the rate of condensation of methylolated kraft lignin is still far less than that for PF resins.

Alonso et al. [325] studied the methylation of softwood and hardwood lignosulfonates by various analytical techniques including UV/vis spectroscopy, FTIR, and <sup>1</sup>H-NMR. With six different samples, under optimal operation conditions, softwood lignosulfonates showed

higher reactivity toward formaldehyde than hardwood lignosulfonates. The synthesized lignin-phenol resin made from methylolated ammonium lignosulfonates was tested. It was found that under optimal conditions, the lignin-phenol resin had similar characteristics to that of the commercial PF resin (reference sample) when 35% of phenol was replaced by methylolated softwood ammonium lignosulfonates.

In the phenolation reaction, the first step involves the protonation of the benzyl hydroxyl group, followed by dehydration at the  $\alpha$ -carbon, to give a carbonium ion. The phenol molecule undergoes an electrophilic attack by carbonium ion giving rise to a phenol condensation product (adduct). After the incorporation of the *ortho*- or *para*-phenyl substituent to the  $\alpha$ -positions of the propanoid sidechains of lignin, adduct fragmentation takes place [326–330]. These steps result in a decrease of MW [331], which favors the incorporation of phenolation products to the resin. Some side reactions can also occur. Thus, depending on the reaction conditions, the carbonium ion can react with a lignosulfonate molecule leading to a self-condensation product [332].

Alonso et al. [322] examined various variables to obtain the optimal phenolation conditions for lignin modification. The characterization of phenolation products was accomplished by GPC, FTIR, and  $^1\text{H-NMR}$ . The optimal phenolation conditions were 120 °C, 160 min, and 30% lignosulfonate content. The  $^1\text{H-NMR}$  analysis showed that the addition of lignosulfonates to the *ortho*-position of phenol predominate over *para*-position addition in adduct formation.

Vazquez et al. [332] used methylolated or phenolated organosolv (acetic acid) lignins from eucalyptus wood to prepare lignin-phenol-formaldehyde resins. Pine and eucalyptus plywood boards manufactured using the resins prepared with the modified lignins complied with the European Standard EN 314-1:1993 for WBP quality boards and gave knife test results similar to those of boards manufactured with a commercial PF resin [330].

There were few studies on the utilization of grass lignins for making lignin-based adhesives found in the literature [334, 335]. However, industrial soda bagasse lignin was shown to have much higher reactivity toward formaldehyde because it has a lower degree of condensation and more phenylpropanoid units (that may be from *p*-coumarate) [336]. Ysbrandy modified autohydrolysis bagasse lignin through phenolation and used the phenolated bagasse lignin to make lignin-based PF resins. The effect of substitution levels of different phenolic components with lignin on physical properties was evaluated by testing of lignin-PF resin impregnated paper laminates. The results from this test showed that the lignin-PF resin with 33% phenolated bagasse lignin produced laminates with better physical properties. Phenolated soda bagasse lignin was recently used to prepare lignin-based PF adhesive. Up to 50% of the phenol can be replaced by soda lignin under optimized conditions where the formulated lignin-based adhesive has

comparable thermal stability [329, 337]. Liu et al. [337] prepared phenolated wheat straw soda lignin and used it to substitute up to 70% of the phenol in formulated PF resin adhesive that was found to have comparable performance to the traditional PF adhesives.

Recent concerns over environmental problems caused by the emission of formaldehyde in the production and use of wood composites bonded with formaldehyde-containing resins promoted the studies on preparing formaldehyde-free wood adhesives [314]. Li and Geng found that polyaminoamide-epichlorohydrin (PAE) is a good cross-linking agent for lignin. A formaldehyde-free wood adhesive system consisting of kraft lignin and PAE resin was developed [339]. Mixing an alkaline kraft lignin solution and a PAE solution produced the lignin-PAE adhesives. The physical prosperities (shear strength and water resistance) of the wood composites bonded with the lignin-PAE adhesives were investigated in detail and the possible reactions between lignin and PAE were discussed. Another formaldehyde-free wood-adhesive system was developed when polyethylenimine (PEI) and kraft lignin was mixed at room temperature [340]. The two-ply plywood bonded with such adhesives by hot pressing (140 °C) for 2–9 min. The best testing results (shear strength and water resistance) were obtained when lignin-PEI resins with a lignin/PEI weight ratio of 2:1 was used. The MW of PEIs in the range of 70,000–150,000 had little effect on the shear strength of the resulting wood composites. In a following study from the same group, a demethylated kraft lignin was used to make lignin-PEI resin adhesive. The cueing mechanism of these lignin-PEI adhesives was proposed to be similar to the quinone-tanning processes in nature [341].

## 6.6.2 Biochemicals/Biofuel from Lignins

Lignin represents a major fraction of biomass (10–30 wt%) and is currently burnt to provide heat in the pulp and paper industry. Bioethanol production from lignocellulosics through the hydrolysis of polysaccharides and the subsequent fermentation of monomer sugars is one of the favorable routes to alleviate our energy dependence on fossil oil [342]. Large quantities of lignins will be produced as byproducts in the future if bioethanol production from lignocellulosics becomes a commercial practice [343]. Therefore, it would be ideal to be able to convert the lignin into higher value fuels or chemicals.

Lignin can be converted into a transportation fuel by dehydroxygenation or zeolite upgrading. Previous dehydroxygenation experiments of lignin feedstock have used sulfided NiMo and CoMo catalysts supported on alumina, chromium, and zeolites at 250–450 °C. Thus, hydrogenation of C=C bonds, hydrogenation of aromatics, and deoxygenation of C–O bonds take place under such



conditions. The major products from dehydroxygenation include phenols, cyclohexanes, benzene, naphthalene, and phenanthrene with liquid oil yields of 61% the original lignin [344–347].

Organosolv (Alcell) lignin was depolymerized in a batch reactor using the Lewis acid catalysts  $\text{NiCl}_2$  and  $\text{FeCl}_3$ . The effects of reaction temperature, time, and catalyst were studied on the conversion of this lignin to gas, solid, and liquid products. The highest yields of ether-soluble monomeric compounds, 30% and 26% with Ni and Fe, respectively, were obtained at 305 °C and 1-h reaction time. Under the reaction conditions studied, both catalysts apparently favor condensation reactions leading to the formation of insoluble residue and low quantities of monomeric compounds were produced [348].

Using HZSM-5 catalyst, Thring et al. [348] studied the direct conversion of solvolytic lignin into both liquid and gaseous hydrocarbon products at over a temperature range of 500–650 °C, and weight hourly space velocities (WHSV) of 2.5–7.5/h. The highest liquid yield was 43%, and the coke and char yields were 15–50%. As the temperature increased, gas yields increased, char and coke yields decreased, and liquid yields decreased. The major liquid components were toluene, benzene, and xylene.

In a recent report, Kleinert and Barth described a novel one-step process for converting lignin into nonviscous organic liquids (oil) [350]. The pyrolysis of lignins achieved in a closed system in the presence of formic acid and an alcohol resulted in complete conversion of the original macromolecular phenylpropanoid structure with the formation of a complex mixture of low-molecular-mass compounds. The majority of these products are aliphatic hydrocarbons, although a substantial number of phenolic structures are also present. The detailed analysis of such bio-oil from lignins has shown that a complete degradation of the lignin takes place irrespective of its origin. The resulting bio-oil has a low-molecular-mass distribution with a preponderance of aliphatic hydrocarbon structures. A substantial number of phenolic compounds are, however, also present, and some of these also contain carboxyl groups. The results clearly show that formic acid is a powerful supplier of atomic hydrogen [351].

Shabtai et al. [351] have developed a two-stage process for conversion of lignin into high-quality reformulated gasoline compositions. In the first stage, a lignin material is subjected to a base-catalyzed depolymerization (BCD) reaction in the presence of a supercritical alcohol as a reaction medium at 320 °C and 120 atm. The alcohol helps maintain supercritical conditions, which help solubilize the lignin. The depolymerization steps break down the lignin into monomeric units. In a recent report, Macala et al. [352] demonstrated that under such supercritical conditions, hydrogenolysis of lignin takes place because methanol disproportionates into hydrogen and carbon dioxide. A model compound treated at such BCD conditions (300 °C,

2 h, methanol as solvent) produced hydrogenolysis instead of hydrolysis products.

In the second stage, the depolymerized lignin product is subjected to a sequential two-step hydroprocessing reaction to produce a reformulated hydrocarbon gasoline product. In the first step, the depolymerized lignin is contacted with a hydrodeoxygenation catalyst to produce a hydrodeoxygenated intermediate product that is hydrogenated in the second step to produce the reformulated hydrocarbon gasoline product containing various desirable naphthenic and paraffinic compounds [352]. The catalysts for these reactors are sulfided NiMo or CoMo catalysts. The products consist of  $\text{C}_7$ – $\text{C}_{11}$  alkylbenzenes,  $\text{C}_5$ – $\text{C}_{11}$  multibranched paraffins, and mono-, di-, tri-, and polyalkylated cyclohexanes and cyclopentanes. Alternatively, the depolymerized lignin could be converted into aryl-methyl ethers by feeding the depolymerized lignin to a reactor for a mild selective C–C hydrocracking treatment (HT) with solid superacid catalysts ( $\text{Pt}/\text{SO}_4/\text{ZrO}_2$  or  $\text{Pt}/\text{WO}_4/\text{ZrO}_2$ ) at 340–375 °C, to completely depolymerize the lignin to monocyclic phenols, and then etherification of the phenols with methanol at 225–275 °C using  $\text{WO}_4/\text{ZrO}_2$ ,  $\text{SO}_4/\text{ZrO}$  or  $\text{SO}_4/\text{MnOx}/\text{Al}_2\text{O}_3$  as catalysts [354].

Yan et al. [354] reported a two-step hydrogenolysis/hydrogenation process for converting lignin or wood materials into hydrocarbons and methanol. In the first step, lignin was hydrogenolyzed in acidic water or aqueous dioxane using Rh/C or Pt/C as catalysts at 473 K and 4 MPa  $\text{H}_2$ . The main hydrogenolysis products from birch lignin are guaiacylpropane, guaiacylpropanol, syringylpropane, and syringylpropanol along with minor dimeric products. The best result was attained when hydrogenolysis of birch wood was performed in 1%  $\text{H}_3\text{PO}_4$  aqueous dioxane (50%) using Pt/C as catalyst producing 46% of monomers and 12% of dimers. In the second step, lignin hydrogenolysis products obtained were converted into the corresponding alkanes and methanol in near quantitative yields. On the basis of the model experiments, Pd/C was the best suitable catalyst for the second hydrogenation/hydrogenolysis of methoxy groups. The alkane ( $\text{C}_8$ – $\text{C}_{17}$  hydrocarbons) products and methanol were produced in 80–90 and 95 mol%, respectively.

## 6.7 CONCLUDING REMARKS

On the basis of all knowledge we have so far, it is clear that lignins in grass plant cell walls are much more complex than those in wood. Several points should be made as follows here:

1. Lignin precursors, monolignols are more diverse in grasses and the biosynthetic pathways for those monolignols are still not quite clear as that for monolignols in woods and dicots. Evidence reported so far suggests that ferulates and acylated monolignols should be considered as authentic lignin monomers.



2. Because of the intimate associations between polysaccharides and lignins in grasses, better procedures for isolating lignins from grass plant cell walls are still needed to better understand structures of grass lignins as well as their cross-linking to polysaccharides. Advancement in this direction will help to engineer plants for benefiting agricultural economics and our society in general.
3. It has been well accepted that ferulates in grasses cross-link polysaccharides and lignins forming so-called lignin-hydroxycinnamate-polysaccharide complex, which severely decrease the digestibility of cell wall polysaccharides by ruminants. The formation of such cross-links has been demonstrated through free radical coupling reactions between ferulates and monolignols or phenolic lignin polymers although the other mechanism cannot be ruled out.
4. Acylated monolignols (acetates, *p*-hydroxybenzoates, and *p*-coumarates) have been demonstrated to be able to participate in a free radical coupling reaction analogously to the three traditional monolignols. Novel tetrahydrofuran structures resulting from lignification using these acylated monolignols have been identified in several lignin preparations including corn lignin. Finding such structures, combined with model studies, verify that lignin acylation results from the involvement of acylated monolignols during lignification.
5. The DFRC method has proven to be very useful in characterization of grass lignins as well as wood lignins because of its advantages. NMR, especially 2D solution-state NMR spectroscopy has been applied to structural analysis of lignins from a variety of origins including grasses. The key to succeed for solution-state NMR characterization of lignins is to have soluble preparations and have good model compounds. Whole-cell-wall dissolution solvent systems developed in our group have proven to be useful for high-resolution solution-state NMR characterization of cell wall components and will undoubtedly play an important role in the characterization of plant cell walls.
6. Utilization of (industrial) lignins has very long history although lignins are still underutilized except for that used as low-value fuel in pulping industry. The situations for grass lignins are much worse because of the complexity and lower availability of grass lignins. However, considering possibly large-scale industrial biorefineries for bioenergy and biochemicals production, large amounts of lignins would become available. Efficient utilization of such lignins will benefit future bioenergy production.

## REFERENCES

- [1] C.F. Delwiche, L.E. Graham, N. Thomson, Lignin like compounds and sporopollenin in Coleochaete, an algal model for land plant ancestry, *Science* 245 (1989) 399–401.
- [2] P.T. Martone, J.M. Estevez, F.C. Lu, K. Ruel, M.W. Denny, C. Somerville, et al., Discovery of lignin in seaweed reveals convergent evolution of cell wall architecture, *Curr. Biol.* 19 (2009) 169–175.
- [3] J.M. Harkin, Lignin a natural polymeric product of phenol oxidation, in: W.I. Taylor, A.R. Battersby (Eds.), *Oxidative Coupling of Phenols*, Marcel Dekker, New York, 1967, pp. 243–321.
- [4] J.M. Harkin, Lignin, in: G.W. Butler (Ed.), *Chemistry and Biochemistry of Herbage*, Academic Press, London, 1973, pp. 323–373.
- [5] T. Higuchi, Lignin biochemistry biosynthesis and biodegradation, *Wood Sci. Technol.* 24 (1990) 23–63.
- [6] R.R. Sederoff, J.J. MacKay, J. Ralph, R.D. Hatfield, Unexpected variation in lignin, *Curr. Opin. Plant Biol.* 2 (1999) 145–152.
- [7] W. Boerjan, J. Ralph, M. Baucher, Lignin biosynthesis, *Annu. Rev. Plant Biol.* 54 (2003) 519–546.
- [8] J. Ralph, K. Lundquist, G. Brunow, F.C. Lu, H. Kim, P.F. Schatz, et al., Lignins: natural polymers from oxidative coupling of 4 hydroxyphenylpropanoids, *Phytochem. Rev.* 3 (2004) 29–60.
- [9] J. Ralph, G. Brunow, P.J. Harris, R.A. Dixon, P.F. Schatz, W. Boerjan, Lignification: are lignins biosynthesized via simple combinatorial chemistry or via proteinaceous control and template replication?, in: F. Daayf, A. El Hadrami, L. Adam, G.M. Ballance (Eds.), *Recent Advances in Polyphenol Research*, Wiley Blackwell Publishing, Oxford, 2008, pp. 36–66.
- [10] K.V. Sarkanen, C.H. Ludwig, Lignins, Occurrence, Formation, Structure and Reactions, Wiley Interscience, New York, 1971.
- [11] E. Sjöström, *Wood Chemistry: Fundamentals and Applications*, Second ed., Academic press, Inc., New York, 1993.
- [12] J.L. McCarthy, A. Islam, Lignin chemistry, technology, and utilization: a brief history, in: W.G. Glasser, R.A. Northey, T.P. Schultz (Eds.), *Lignin: Historical, Biological, and Materials Perspectives*, ACS, Washington, DC, 2000, pp. 2–99.
- [13] H.I. Bolker, Lignins, in: *Natural and Synthetic Polymers*, An Introduction, Marcel Dekker, New York, 1974, pp. 577–621.
- [14] R.F. Gould, *Lignin Structure and Reactions*, Am. Chem. Soc., Washington, DC, 1966.
- [15] E. Adler, Lignin chemistry past, present and future, *Wood Sci. Technol.* 11 (1977) 169–218.
- [16] C. Chen, Lignins: occurrence in woody tissues, isolation, reactions, and structure, in: M. Lewin, I.S. Goldstein (Eds.), *Wood Structure and Composition*, Marcel Dekker, New York, 1991, pp. 183–261.
- [17] A. Sakakibara, Chemistry of lignin, in: D.N.S. Hon, N. Shiraishi (Eds.), *Wood and Cellulosic Chemistry*, Marcel Dekker, New York, 1991, pp. 113–175.
- [18] S.Y. Lin, C.W. Dence, *Methods in Lignin Chemistry*, Springer Verlag, Heidelberg, 1992.
- [19] N.G. Lewis, A 20th century roller coaster ride: a short account of lignification, *Curr. Opin. Plant Biol.* 2 (1999) 153–162.
- [20] A.M. Boudet, Lignins and lignification: selected issues, *Plant Physiol. Bioch.* 38 (2000) 81–96.
- [21] B. Monties, Botanical variability and mechanical function of lignins: two critical aspects of the plant phenolic secondary metabolism, in: F. Imperato (Ed.), *Advances in Phytochemistry, Research Signpost, Kerala*, 2003, pp. 1–48.
- [22] M. Baucher, C. Halpin, M. Petit Conil, W. Boerjan, Lignin: genetic engineering and impact on pulping, *Crit. Rev. Biochem. Mol.* 38 (2003) 305–350.
- [23] A.U. Buranov, G. Mazza, Lignin in straw of herbaceous crops, *Ind. Crop. Prod.* 28 (2008) 237–259.

- [24] S. Kim, B.E. Dale, Global potential bioethanol production from wasted crops and crop residues, *Biomass Bioenerg.* 26 (2004) 361–375.
- [25] J.H. Grabber, R.D. Hatfield, F.C. Lu, J. Ralph, Coniferyl ferulate incorporation into lignin enhances the alkaline delignification and enzymatic degradation of cell walls, *Biomacromolecules* 9 (2008) 2510–2516.
- [26] J.H. Grabber, How do lignin composition, structure, and cross linking affect degradability? a review of cell wall model studies, *Crop Sci.* 45 (2005) 820–831.
- [27] H. Kim, J. Ralph, N. Yahiaoui, M. Pean, A.M. Boudet, Cross coupling of hydroxycinnamyl aldehydes into lignins, *Org. Lett.* 2 (2000) 2197–2200.
- [28] J. Ralph, C. Lapierre, F.C. Lu, J.M. Marita, G. Pilate, J. Van Doorselaere, et al., NMR evidence for benzodioxane structures resulting from incorporation of 5 hydroxyconiferyl alcohol into lignins of *O* methyltransferase deficient poplars, *J. Agr. Food Chem.* 49 (2001) 86–91.
- [29] J. Ralph, C. Lapierre, J.M. Marita, H. Kim, F.C. Lu, R.D. Hatfield, et al., Elucidation of new structures in lignins of CAD and COMT deficient plants by NMR, *Phytochemistry* 57 (2001) 993–1003.
- [30] H. Kim, J. Ralph, F.C. Lu, S.A. Ralph, A.M. Boudet, J.J. MacKay, et al., NMR analysis of lignins in CAD deficient plants. Part 1. Incorporation of hydroxycinnamaldehydes and hydroxybenzaldehydes into lignins, *Org. Biomol. Chem.* 1 (2003) 268–281.
- [31] C. Lapierre, G. Pilate, B. Pollet, I. Mila, J.C. Leple, L. Jouanin, et al., Signatures of cinnamyl alcohol dehydrogenase deficiency in poplar lignins, *Phytochemistry* 65 (2004) 313–321.
- [32] F.C. Lu, J. Ralph, Novel tetrahydrofuran structures derived from  $\beta$   $\beta$  coupling reactions involving sinapyl acetate in Kenaf lignins, *Org. Biomol. Chem.* 6 (2008) 3681–3694.
- [33] F.C. Lu, J. Ralph, Novel  $\beta$   $\beta$  structures in natural lignins incorporating acylated monolignols, in: *Thirteenth International Symposium on Wood, Fiber, and Pulping Chemistry*, APPITA, Australia, Auckland, New Zealand, 2005, pp. 233–237.
- [34] R. Vanholme, K. Morreel, J. Ralph, W. Boerjan, Lignin engineering, *Curr. Opin. Plant Biol.* 11 (2008) 278–285.
- [35] J. Ralph, H. Kim, F.C. Lu, J.H. Grabber, J.C. Leple, J. Berrio Sierra, et al., Identification of the structure and origin of a thioacidolysis marker compound for ferulic acid incorporation into angiosperm lignins (and an indicator for cinnamoyl CoA reductase deficiency), *Plant J.* 53 (2008) 368–379.
- [36] M. Baucher, B. Monties, M. Van Montagu, W. Boerjan, Biosynthesis and genetic engineering of lignin, *Crit. Rev. Plant Sci.* 17 (1998) 125–197.
- [37] H.G. Teutsch, M.P. Hasenfratz, A. Lesot, C. Stoltz, J.M. Garnier, J. M. Jeltsch, et al., Werckreichhart, Isolation and sequence of a cDNA encoding the Jerusalem artichoke cinnamate 4 hydroxylase, a major plant cytochrome P450 involved in the general phenylpropanoid pathway, *Proc. Natl. Acad. Sci. U S A* 90 (1993) 4102–4106.
- [38] R. Franke, J.M. Humphreys, M.R. Hemm, J.W. Denault, M. O. Ruegger, J.C. Cusumano, et al., The Arabidopsis REF8 gene encodes the 3 hydroxylase of phenylpropanoid metabolism, *Plant J.* 30 (2002) 33–45.
- [39] G. Schoch, S. Goepfert, M. Morant, A. Hehn, D. Meyer, P. Ullmann, et al., CYP98A3 from Arabidopsis thaliana is a 3' hydroxylase of phenolic esters, a missing link in the phenylpropanoid pathway, *J. Biol. Chem.* 276 (2001) 36566–36574.
- [40] R.B. Nair, Q. Xia, C.J. Kartha, E. Kurylo, R.N. Hirji, R. Datla, et al., Arabidopsis CYP98A3 mediating aromatic 3 hydroxylation. Developmental regulation of the gene, and expression in yeast, *Plant Physiol.* 130 (2002) 210–220.
- [41] B. Ulbrich, M.H. Zenk, Partial purification and properties of *p* hydroxycinnamoyl CoA: shikimate *p* hydroxycinnamoyl transferase from higher plants, *Phytochemistry* 19 (1980) 1625–1629.
- [42] L. Hoffmann, S. Maury, F. Martz, P. Geoffroy, M. Legrand, Purification, cloning, and properties of an acyltransferase controlling shikimate and quinate ester intermediates in phenylpropanoid metabolism, *J. Biol. Chem.* 278 (2003) 95–103.
- [43] J.M. Humphreys, M.R. Hemm, C. Chapple, New routes for lignin biosynthesis defined by biochemical characterization of recombinant ferulate 5 hydroxylase, a multifunctional cytochrome P450 dependent monooxygenase, *Proc. Natl. Acad. Sci. U S A* 96 (1999) 10045–10050.
- [44] K. Osakabe, C.C. Tsao, L.G. Li, J.L. Popko, T. Umezawa, D. T. Carraway, et al., Coniferyl aldehyde 5 hydroxylation and methylation direct syringyl lignin biosynthesis in angiosperms, *Proc. Natl. Acad. Sci. U S A* 96 (1999) 8955–8960.
- [45] L. Li, J.L. Popko, T. Umezawa, V.L. Chiang, 5 Hydroxyconiferyl aldehyde modulates enzymatic methylation for syringyl monolignol formation, a new view of monolignol biosynthesis in angiosperms, *J. Biol. Chem.* 275 (2000) 6537–6545.
- [46] K. Parvathi, F. Chen, D.J. Guo, J.W. Blount, R.A. Dixon, Substrate preferences of *O* methyltransferases in alfalfa suggest new pathways for 3 *O* methylation of monolignols, *Plant J.* 25 (2001) 193–202.
- [47] J. Ralph, M. Bunzel, J.M. Marita, R.D. Hatfield, F.C. Lu, H. Kim, et al., Peroxidase dependent cross linking reactions of *p* hydroxycinnamates in plant cell walls, *Phytochem. Rev.* 3 (2004) 79–96.
- [48] L.A. Donaldson, Lignification and lignin topochemistry an ultra structural view, *Phytochemistry* 57 (2001) 859–873.
- [49] N. Terashima, K. Fukushima, L.F. He, K. Takabe, Comprehensive model of the lignified plant cell wall, in: H.G. Jung, D.R. Buxton, R.D. Hatfield, J. Ralph (Eds.), *Forage Cell Wall Structure and Digestibility*, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, 1993, pp. 247–270.
- [50] F.M. Engels, J.L.L. Schuurmans, Relationship between structural development of cell walls and degradation of tissues in maize stems, *J. Sci. Food Agr.* 59 (1992) 45–51.
- [51] F.M. Engels, H.G. Jung, Alfalfa stem tissues: cell wall development and lignification, *Ann. Bot.* 82 (1998) 561–568.
- [52] C. Vallet, B. Chabbert, Y. Czaninski, B. Monties, Histochemistry of lignin deposition during sclerenchyma differentiation in alfalfa stems, *Ann. Bot.* 78 (1996) 625–632.
- [53] S. Saka, D.A.I. Goring, Localization of lignins in wood cell walls, in: T. Higuchi (Ed.), *Biosynthesis and Biodegradation of Wood Components*, Academic Press, Orlando, 1985, pp. 51–62.
- [54] G. Musel, T. Schindler, R. Bergfeld, K. Ruel, G. Jacquet, C. Lapierre, et al., Structure and distribution of lignin in primary and secondary cell walls of maize coleoptiles analyzed by chemical and immunological probes, *Planta* 201 (1997) 146–159.
- [55] M. Chabannes, K. Ruel, A. Yoshinaga, B. Chabbert, A. Jauneau, J. P. Joseleau, et al., In situ analysis of lignins in transgenic tobacco reveals a differential impact of individual transformations on the spatial patterns of lignin deposition at the cellular and subcellular levels, *Plant J.* 28 (2001) 271–282.
- [56] J.P. Joseleau, K. Ruel, Study of lignification by noninvasive techniques in growing maize internodes: An investigation by Fourier transform infrared cross polarization magic angle spinning  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy and immunocytochemical

- transmission electron microscopy, *Plant Physiol.* 114 (1997) 1123 1133.
- [57] F.C. Lu, J. Ralph, Detection and determination of *p* coumaroylated units in lignins, *J. Agr. Food Chem.* 47 (1999) 1988 1992.
- [58] L.F. He, N. Terashima, Formation and structure of lignin in monocotyledons. 4. Deposition process and structural diversity of the lignin in the cell wall of sugarcane and rice plant studied by ultraviolet microscopic spectroscopy, *Holzforschung* 45 (1991) 191 198.
- [59] K. Freudenberg, Lignin: its constitution and formation from *p* hydroxycinnamyl alcohols, *Science* 148 (1965) 595 600.
- [60] F. Chioccare, S. Poli, B. Rindone, T. Pilati, G. Brunow, P. Pietikainen, et al., Regioselective and diastereoselective synthesis of dimeric lignans using oxidative coupling, *Acta Chem. Scand.* 47 (1993) 610 616.
- [61] S. Quideau, J. Ralph, A biomimetic route to lignin model compounds via silver (I) oxide oxidation. 1. Synthesis of dilignols and non cyclic benzyl aryl ethers, *Holzforschung* 48 (1994) 12 22.
- [62] L.L. Landucci, S. Luque, S. Ralph, Reaction of *p* hydroxycinnamyl alcohols with transition metal salts. 2. Preparation of guaiacyl/syringyl dilignols, trilignols, and tetralignols, *J. Wood Chem. Technol.* 15 (1995) 493 513.
- [63] F. Nakatsubo, Enzymic dehydrogenation of *p* coumaryl alcohol and syntheses of oligolignols, *Wood Res.* 67 (1981) 59 118.
- [64] R. Hatfield, W. Vermerris, Lignin formation in plants. The dilemma of linkage specificity, *Plant Physiol.* 126 (2001) 1351 1357.
- [65] A.P. Zhang, F.C. Lu, R.C. Sun, J. Ralph, Ferulate coniferyl alcohol cross coupled products formed by radical coupling reactions, *Planta* 229 (2009) 1099 1108.
- [66] G. Jacquet, B. Pollet, C. Lapierre, New ether linked ferulic acid coniferyl alcohol dimers identified in grass straws, *J. Agr. Food Chem.* 43 (1995) 2746 2751.
- [67] M. Bunzel, J. Ralph, F.C. Lu, R.D. Hatfield, H. Steinhart, Lignins and ferulate coniferyl alcohol cross coupling products in cereal grains, *J. Agr. Food Chem.* 52 (2004) 6496 6502.
- [68] J. Ralph, R.F. Helm, Lignin/hydroxycinnamic acid/polysaccharide complexes: Synthetic models for regiochemical characterization, in: H.G. Jung, D.R. Buxton, R.D. Hatfield, J. Ralph (Eds.), *Forage Cell Wall Structure and Digestibility*, ASA ACSSA SSSA, Madison, 1993, pp. 201 246.
- [69] J. Ralph, R.D. Hatfield, J.H. Grabber, H.G. Jung, S. Quideau, R.F. Helm, Cell wall cross linking in grasses by ferulates and diferulates, in: N.G. Lewis, S. Sarkanen, (Eds.), *Lignin and Lignan Biosynthesis*, American Chemical Society, Washington, DC, 1998, pp. 209 236.
- [70] J.H. Grabber, J. Ralph, R.D. Hatfield, Ferulate cross links limit the enzymatic degradation of synthetically lignified primary walls of maize, *J. Agr. Food Chem.* 46 (1998) 2609 2614.
- [71] J.H. Grabber, R.D. Hatfield, J. Ralph, Diferulate cross links impede the enzymatic degradation of non lignified maize walls, *J. Sci. Food Agr.* 77 (1998) 193 200.
- [72] C.L. Willms, L.L. Berger, N.R. Merchen, G.C. Fahey, Utilization of alkaline hydrogen peroxide treated wheat straw in cattle growing and finishing diets, *J. Anim. Sci.* 69 (1991) 3917 3924.
- [73] M. Amjed, H.G. Jung, J.D. Donker, Effect of alkaline hydrogen peroxide treatment on cell wall composition and digestion kinetics of sugarcane residues and wheat straw, *J. Anim. Sci.* 70 (1992) 2877 2884.
- [74] J. Ralph, R.D. Hatfield, S. Quideau, R.F. Helm, J.H. Grabber, H.J.G. Jung, Pathway of *p* coumaric acid incorporation into maize lignin as revealed by NMR, *J. Am. Chem. Soc.* 116 (1994) 9448 9456.
- [75] Y.Z. Lai, K.V. Sarkanen, Isolation and structural studies, in: K.V. Sarkanen, C.H. Ludwig (Eds.), *Lignins: Occurrence, Formation, Structure and Reactions*, Wiley Interscience, New York, 1971, pp. 165 230.
- [76] J.R. Obst, T.K. Kirk, Isolation of lignin, in: W.A. Wood, S. T. Kellogg (Eds.), *Methods in Enzymology, Biomass Part B*, Academic Press, New York, 1988, pp. 3 12.
- [77] H.M. Chang, E.B. Cowling, W. Brown, E. Adler, G. Miksche, Comparative studies on cellulolytic enzyme lignin and milled wood lignin of sweetgum and spruce, *Holzforschung* 29 (1975) 153 159.
- [78] A. Björkman, Studies on finely divided wood. Part I. Extraction of lignin with neutral solvents, *Sven. Papperstidn.* 59 (1956) 477 485.
- [79] A. Björkman, Isolation of lignin from finely divided wood with neutral solvents, *Nature* 174 (1954) 1057 1058.
- [80] Z. Hu, T.F. Yeh, H.M. Chang, Y. Matsumoto, J.F. Kadla, Elucidation of the structure of cellulolytic enzyme lignin, *Holz forschung* 60 (2006) 389 397.
- [81] P. Whiting, D.A.I. Goring, The morphological origin of milled wood lignin, *Sven. Papperstidn.* 84 (1981) 120 122.
- [82] N. Terashima, K. Fukushima, T. Imai, Morphological origin of milled wood lignin studied by radiotracer method, *Holzforschung* 46 (1992) 271 275.
- [83] K. Lundquist, Wood, in: S.Y. Lin, C.W. Dence (Eds.), *Methods in Lignin Chemistry*, Springer Verlag, 1992, pp. 65 70.
- [84] A. Björkman, B. Person, Studies on finely divided wood. Part 2. Extraction of lignin with neutral solvents, *Sven. Papperstidn.* 60 (1957) 158 169.
- [85] D.E. Bland, Colorimetric and chemical identification of lignins in different parts of eucalyptus botryoides and their relation to lignification, *Holzforschung* 20 (1966) 12 21.
- [86] K.M. Holtman, H.M. Chang, J.F. Kadla, An NMR comparison of the whole lignin from milled wood, MWL, and REL dissolved by the DMSO/NMI procedure, *J. Wood Chem. Technol.* 27 (2007) 179 200.
- [87] A. Maurer, D. Fengel, On the origin of milled wood lignin. 1. The influence of ball milling on the ultrastructure of wood cell walls and the solubility of lignin, *Holzforschung* 46 (1992) 417 423.
- [88] T. Ikeda, K. Holtman, J.F. Kadla, H. Chang, H. Jameel, Studies on the effect of ball milling on lignin structure using a modified DFRC method, *J. Agr. Food Chem.* 50 (2002) 129 135.
- [89] A. Fugimoto, Y. Matsumoto, H. Chang, G. Meshitsuka, Quantitative evaluation of milling effects on lignin structure during the isolation process of milled wood lignin, *J. Wood Sci.* 51 (2005) 89 91.
- [90] T. Higuchi, Y. Ito, M. Shimada, I. Kawamura, Chemical properties of milled wood lignin of grasses, *Phytochemistry* 6 (1967) 1551 1556.
- [91] A. Scalbert, B. Monties, J.Y. Lallemand, E. Guittet, C. Rolando, Ether linkage between phenolic acids and lignin fractions from wheat straw, *Phytochemistry* 24 (1985) 1359 1362.
- [92] J. Ralph, J.H. Grabber, R.D. Hatfield, Lignin ferulate cross links in grasses Active incorporation of ferulate polysaccharide esters into ryegrass lignins, *Carbohydr. Res.* 275 (1995) 167 178.
- [93] C. Crestini, D.S. Argyropoulos, Structural analysis of wheat straw lignin by quantitative P 31 and 2D NMR spectroscopy. The occurrence of ester bonds and alpha O 4 substructures, *J. Agr. Food Chem.* 45 (1997) 1212 1219.
- [94] H.J.G. Jung, D.S. Himmelsbach, Isolation and characterization of wheat straw lignin, *J. Agr. Food Chem.* 37 (1989) 81 87.
- [95] J.C. Pew, Properties of powdered wood and isolation of lignin by cellulytic enzymes, *Tappi.* 40 (1957) 553 558.

- [96] J.C. Pew, P. Weyna, Fine grinding, enzyme digestion, and the lignin cellulose bond in wood, *Tappi* 45 (1962) 247–256.
- [97] T. Iversen, S. Wännström, Lignin carbohydrate bonds in a residual lignin isolated from pine kraft pulp, *Holzforschung* 40 (1986) 19–22.
- [98] Y. Chen, Y. Shimizu, M. Takai, J. Hayashi, A method for isolation of milled wood lignin involving solvent swelling prior to enzyme treatment, *Wood Sci. Technol.* 29 (1995) 295–306.
- [99] S. Wu, D. Argyropoulos, An improved method for isolating lignin in high yield and purity, *J. Pulp Pap. Sci.* 29 (2003) 235–239.
- [100] A. Guerra, I. Filpponen, L. Lucia, C. Saquing, S. Baumberger, D.S. Argyropoulos, Toward a better understanding of the lignin isolation process from wood, *J. Agr. Food Chem.* 54 (2006) 5939–5947.
- [101] A. Guerra, I. Filpponen, L.A. Lucia, D.S. Argyropoulos, Comparative evaluation of three lignin isolation protocols for various wood species, *J. Agr. Food Chem.* 54 (2006) 9696–9705.
- [102] X.A. Liu, Z.Z. Lee, D.S. Tai, Fractional studies on the characteristics of high alkali soluble lignins of wheat straw, *Cell. Chem. Technol.* 23 (1989) 559–572.
- [103] M.C. Terrón, A.M. Calvo, M.L. Fidalgo, M. Manzanares, M. Ballesteros, A.T. Martínez, et al., Chemical characterization and fungal decolorization of straw soda pulping effluents, in: M. Kuwahara, M. Shimada (Eds.), *Biotechnology in Pulp and Paper Industry*, Uni publishers Co. Ltd., Tokyo, 1992, pp. 51–56.
- [104] C. Lapiere, D. Jouin, B. Monties, On the molecular origin of the alkali solubility of Gramineae lignins, *Phytochemistry* 28 (1989) 1401–1402.
- [105] T. Higuchi, Y. Ito, I. Kawamura, *p* Hydroxyphenylpropane component of grass lignin and roles of tyrosine ammonia lyase in its formation, *Phytochemistry* 6 (1967) 875–881.
- [106] K. Iiyama, T.B.T. Lam, B.A. Stone, Phenolic acid bridges between polysaccharides and lignin in wheat straw internodes, *Phytochemistry* 29 (1990) 733–737.
- [107] T.B.T. Lam, K. Iiyama, B.A. Stone, Lignin in wheat straw internodes. Part 2: Alkaline nitrobenzene oxidation of wheat straw lignin and its fractions, *J. Sci. Food Agr.* 51 (1990) 493–506.
- [108] A. Scalbert, B. Monties, Composition of wheat straw lignin preparations, *Holzforschung* 40 (1986) 249–254.
- [109] R.C. Sun, J.M. Lawther, W.B. Banks, Influence of alkali pretreatment on wheat straw cell wall components, *Ind. Crop. Prod.* 4 (1995) 127–145.
- [110] R.C. Sun, J.M. Lawther, W.B. Banks, Fractional isolation and physico chemical characterization of alkali soluble lignins from wheat straw, *Holzforschung* 51 (1997) 244–250.
- [111] R.C. Sun, J.M. Lawther, W.B. Banks, The effect of alkaline nitrobenzene oxidation conditions on the yield and components of phenolic monomers in wheat straw lignin and compared to cupric (II) oxidation, *Ind. Crop. Prod.* 4 (1995) 241–254.
- [112] R.C. Sun, J.M. Lawther, W.B. Banks, Effects of extraction time and different alkalis on the composition of alkali soluble wheat straw lignins, *J. Agr. Food Chem.* 144 (1996) 3965–3970.
- [113] R.C. Sun, J.M. Lawther, W.B. Banks, Effects of pretreatment temperature and alkali concentration on the composition of alkali soluble lignins from wheat straw, *J. Appl. Polym. Sci.* 62 (1996) 1473–1481.
- [114] R.C. Sun, J.M. Lawther, W.B. Banks, A tentative chemical structure of wheat straw lignin, *Ind. Crop. Prod.* 6 (1997) 1–8.
- [115] R.C. Sun, P. Rowlands, J.M. Lawther, Rapid isolation and physico chemical characterization of wheat straw lignins, *Recent Res. Devel. Agr. Food Chem.* 4 (2000) 1–26.
- [116] C.W. Dence, S. Omori, A survey of hydrogen peroxide bleaching of mechanical and chemimechanical pulpfactors affecting brightness, *Tappi* J. 69 (1986) 120–125.
- [117] J. Gierer, Chemistry of delignification Part 2: reactions of lignins during bleaching, *Wood Sci. Technol.* 20 (1986) 1–33.
- [118] G.X. Pan, J.L. Bolton, G.J. Leary, Determination of ferulic and *p* coumaric acids in wheat straw and the amounts released by mild acid and alkaline peroxide treatment, *J. Agr. Food Chem.* 46 (1988) 5283–5288.
- [119] R. Agnemo, G. Gellerstedt, The reactions of lignin with alkaline hydrogen peroxide, 2: Factors influencing the decomposition of phenolic structures, *Acta Chem. Scand.* 33 (1979) 337–342.
- [120] J.M. Gould, Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification, *Biotechnol. Bioeng.* 26 (1984) 46–52.
- [121] J.M. Gould, Studies on the mechanism of alkaline peroxide delignification of agricultural residues, *Biotechnol. Bioeng.* 27 (1985) 225–231.
- [122] H.U. Süß, W. Eul, O. Helmling, Semibleaching of kraft pulp using oxygen and hydrogen peroxide, *Jahrgang Heft* 43 (1989) 318–323.
- [123] D. Lachenal, C.D. Choudens, P. Monzie, Hydrogen peroxide as a delignifying agent, *Tappi* 63 (1980) 119–122.
- [124] G.V. Rao, K. Gopichand, A.V. Rao, C.V. Swamy, Brightness reversion and chemical properties of bleached pulp in short sequence bleaching with hydrogen peroxide, *Cell. Chem. Technol.* 25 (1991) 391–395.
- [125] M.S. Kerley, G.C. Fahey Jr., L.L. Berger, J.M. Gould, F.L. Baker, Alkaline hydrogen peroxide treatment unlocks energy in agricultural by products, *Science* 230 (1985) 820–822.
- [126] J.M. Gould, S.N. Freer, High efficiency ethanol production from lignocellulosic residues pretreated with alkaline H<sub>2</sub>O<sub>2</sub>, *Biotechnol. Bioeng.* 26 (6) (1984) 628–631.
- [127] B. Yan, A. Boussaid, S.D. Mansfields, D.J. Gregg, J.N. Saddler, Fast and efficient alkaline peroxide pretreatment to enhance the enzymatic digestibility of steam exploded softwood substrates, *Biotechnol. Bioeng.* 77 (6) (2002) 678–684.
- [128] B.C. Saha, M.A. Cotta, Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw, *Biotechnol. Progr.* 22 (2) (2006) 449–453.
- [129] M.J. Selig, T.B. Vinzant, M.E. Himmel, S.R. Decker, The Effect of lignin removal by alkaline peroxide pretreatment on the susceptibility of corn stover to purified cellulolytic and xylanolytic enzymes, *Appl. Biochem. Biotech.* 155 (1–3) (2009) 397–406.
- [130] D. Lachenal, J. Papadopoulos, Improvement of hydrogen peroxide delignification, *Cell. Chem. Technol.* 22 (1988) 537–546.
- [131] T.J. McDonough, The chemistry of organosolv delignification, *Tappi* J. 76 (1993) 186–193.
- [132] K.V. Sarkanen, L.H. Hoo, Kinetics of hydrolysis of *erythro* guaiacylglycerol  $\beta$  (2-methoxyphenyl) ether and its veratryl analogue using HCl and aluminum chloride as catalysts, *J. Wood Chem. Technol.* 1 (1981) 11–27.
- [133] J.M. Lawther, R.C. Sun, W.B. Banks, Extraction and comparative characterization of ball milled lignin LM, enzyme lignin LE and alkali lignin LA from wheat straw, *Cellul. Chem. Technol.* 30 (1996) 395–410.
- [134] H. Vázquez Torres, G. Canché Escamilla, C.A. Cruz Ramos, Coconut husk lignin. I. Extraction and characterization, *J. Appl. Polym. Sci.* 45 (1992) 633–644.
- [135] H. Vázquez Torres, G. Canché Escamilla, C.A. Cruz Ramos, Coconut husk lignin. II. Characterization by infrared and nuclear



- magnetic resonance spectroscopy, *J. Appl. polym. Sci.* 45 (1992) 645–653.
- [136] J.H. Lora, S. Aziz, Organosolv pulping: a versatile approach to wood refining, *Tappi J.* 68 (1985) 94–97.
- [137] H.H. Nimz, R. Casten, Chemical processing of lignocellulosics, *Holz Roh. Werkst.* 44 (1986) 207–212.
- [138] Y. Sano, M. Nakamura, S. Shimamoto, Pulping of wood at atmospheric pressure II: pulping of hardwoods with aqueous acetic acid containing a small amount of sulfuric acid, *Mokuzai Gakkaishi* 36 (1990) 207–211.
- [139] X.J. Pan, Y. Sano, Comparison of acetic acid lignin with milled wood and alkaline lignins from wheat straw, *Holzforschung* 54 (2000) 61–65.
- [140] F. Xu, J.X. Sun, R.C. Sun, P. Fowler, M.S. Baird, Comparative study of organosolv lignins from wheat straw, *Ind. Crop. Prod.* 23 (2006) 180–193.
- [141] D. Gast, C. Ayla, J. Puls, Component separation of lignocelluloses by organosolv treatment, *Proc. 2nd Energy from Biomass Conf.*, Berlin, Germany (1982), pp. 879–882.
- [142] D. Gast, J. Puls, Improvement of the ethylene glycerol water system for the component separation of lignocelluloses, *Proc. 3rd Energy from Biomass Conf.*, Venice, Italy (1985) 949–952.
- [143] B.D. Bogomolov, A.S. Groshev, A.P. Vishnyakova, Wood delignification with tetrahydrofurfuryl alcohol. 1. Comparison of delignification capacity of tetrahydrofurfuryl alcohol and other organic solvents, *Khim. Drev. (Riga)* 4 (1979) 21–24.
- [144] J. Kajimoto, W.E. Widodo, T. Kishimoto, Y. Uraki, Y. Sano, HBS pulping (1) pulping of softwood, *Japan Tappi*, 54 (2000) 1252–1259.
- [145] W.E. Widodo, J. Kajimoto, Y. Sano, HBS pulping (2) pulping of hardwood and annual plants, *Japan Tappi*, 54 (2000) 1394–1402.
- [146] T. Kishimoto, Y. Sano, Delignification mechanism during high boiling solvent pulping Part 1. Reaction of guaiacylglycerol  $\beta$  guaiacyl ether, *Holzforschung* 55 (2001) 611–616.
- [147] T. Kishimoto, Y. Sano, Delignification mechanism during high boiling solvent pulping Part 2. Homolysis of guaiacylglycerol  $\beta$  guaiacyl ether, *Holzforschung* 56 (2002) 623–631.
- [148] T. Kishimoto, A. Ueki, Y. Sano, Delignification mechanism during high boiling solvent pulping Part 3. Structural changes in lignin analyzed by  $^{13}\text{C}$  NMR spectroscopy, *Holzforschung* 57 (2003) 602–610.
- [149] T. Kishimoto, A. Ueki, H. Takamori, Y. Uraki, M. Ubukata, Delignification mechanism during high boiling solvent pulping. Part 6: Changes in lignin structure analyzed by  $^1\text{H}$   $^{13}\text{C}$  correlation 2D NMR spectroscopy, *Holzforschung* 58 (2004) 355–362.
- [150] T. Kishimoto, Y. Sano, Delignification mechanism during high boiling solvent pulping. IV. Effect of a reducing sugar on the degradation of guaiacylglycerol  $\beta$  guaiacyl ether, *J. Wood Chem. Technol.* 23 (2003) 233–248.
- [151] T. Kishimoto, Y. Sano, Delignification mechanism during high boiling solvent pulping. V. Reaction of nonphenolic  $\beta$  O 4 model compounds in the presence and absence of glucose, *J. Wood Chem. Technol.* 23 (2003) 279–292.
- [152] E. Adler, G. Brunow, K. Lundquist, Investigation of the acid catalyzed alkylation of lignins by means of NMR spectroscopic methods, *Holzforschung* 41 (1987) 199–207.
- [153] K. Lundquist, R. Simonson, K. Tingsvik, On the occurrence of carbohydrates in milled wood lignin preparations, *Sven. Papperstidn.* 82 (1979) 272–275.
- [154] K. Lundquist, R. Simonson, K. Tingsvik, Studies on lignin carbohydrate linkages in milled wood lignin preparations, *Sven. Papperstidn.* 83 (1980) 452–454.
- [155] C. Schuerch, The solvent properties of liquids and their relation to the solubility, swelling, isolation and fractionation of lignin, *J. Am. Chem. Soc.* 74 (1952) 5061–5067.
- [156] J.J. Lindberg, H. Tylli, C. Majani, Notes on the molecular weight and the fractionation of lignins with organic solvents, *Paperi Puu* 46 (1964) 521–526.
- [157] S.Y. Lin, W.J. Detroit, Chemical heterogeneity of technical lignins its significance in lignin utilization, *International Symposium on Wood and Pulping Chemistry, The Ekman days, IV* (1981), 44–50.
- [158] R. Mörck, H. Yoshida, K.P. Kringstad, Fractionation of kraft lignin by successive extraction with organic solvents. 1. Functional groups,  $^{13}\text{C}$  NMR spectra and molecular weight distributions, *Holz forschung* 40 (1986) Suppl. 51–60.
- [159] R. Mörck, A. Reimann, K.P. Kringstad, Fractionation of kraft lignin by successive extraction with organic solvents. III. Fractionation of kraft lignin from birch, *Holzforchung* 42 (1988) 111–116.
- [160] R.C. Sun, J. Tomkinson, G.L. Jones, Fractional characterization of ash AQ lignin by successive extraction with organic solvents from oil palm EFB fibre, *Polym. Degrad. Stabil.* 68 (2000) 111–119.
- [161] R.C. Sun, J. Tomkinson, S. Griffiths, Fractional and physico chemical analysis of soda AQ lignin by successive extraction with organic solvents from oil palm EFB fiber, *Int. J. Polym. Anal. Ch.* 5 (2000) 531–547.
- [162] T.Q. Yuan, J. He, F. Xu, R.C. Sun, Fractionation and physico chemical analysis of degraded lignins from the black liquor of *Eucalyptus pellita* KP AQ pulping, *Polym. Degrad. Stabil.* 94 (2009) 1142–1150.
- [163] R. Alén, P. Patja, E. Sjöström, Carbon dioxide precipitation of lignin from pine kraft black liquor, *Tappi* 62 (1979) 108–110.
- [164] H. Loufti, B. Blackwell, V. Uloth, Lignin recovery from kraft black liquor: preliminary process design, *Tappi J.* 74 (1991) 203–210.
- [165] R.A. Northey, Low cost uses of lignin, *ACS Symp. Ser.* 476 (1992) 146–175.
- [166] M.A. Gilarranz, F. Rodriguez, M. Oliet, J.A. Revenga, Acid precipitation and purification of wheat straw lignin, *Sep. Sci. Technol.* 33 (1998) 1359–1377.
- [167] R.C. Sun, J. Tomkinson, J. Bolton, Separation and characterization of lignins from the black liquor of oil palm trunk fiber pulping, *Sep. Sci. Technol.* 35 (1999) 3045–3058.
- [168] C. Lapiere, B. Monties, C. Rolando, Preparative thioacidolysis of spruce lignin isolation and identification of main monomeric products, *Holzforchung* 40 (1986) 47–50.
- [169] C. Lapiere, B. Monties, C. Rolando, Thioacidolysis of poplar lignins identification of monomeric syringyl products and characterization of guaiacyl syringyl lignin fractions, *Holzforchung* 40 (1986) 113–118.
- [170] C. Lapiere, B. Monties, C. Rolando, Thioacidolysis of lignin comparison with acidolysis, *J. Wood Chem. Technol.* 5 (1985) 277–292.
- [171] C. Lapiere, Application of new methods for the investigation of lignin structure, in: H.G. Jung, D.R. Buxton, R.D. Hatfield, J. Ralph (Eds.), *Forage Cell Wall Structure and Digestibility*, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, 1993, pp. 133–166.
- [172] C. Lapiere, B. Pollet, B. Monties, C. Rolando, Thioacidolysis of spruce lignin GC MS analysis of the main dimers recovered after raney nickel desulfuration, *Holzforchung* 45 (1991) 61–68.
- [173] H. Onnerud, G. Gellerstedt, Inhomogeneities in the chemical structure of spruce lignin, *Holzforchung* 57 (2003) 165–170.
- [174] H. Onnerud, G. Gellerstedt, Inhomogeneities in the chemical structure of hardwood lignins, *Holzforchung* 57 (2003) 255–265.



- [175] C. Lapiere, C. Rolando, Thioacidolyses of pre methylated lignin samples from pine compression and poplar woods, *Holzforschung* 42 (1988) 1 4.
- [176] C. Lapiere, B. Monties, C. Rolando, Thioacidolyses of diazo methane methylated pine compression wood and wheat straw insitu lignins, *Holzforschung* 42 (1988) 409 411.
- [177] J.H. Grabber, S. Quideau, J. Ralph, *p* coumaroylated syringyl units in maize lignin: Implications for beta ether cleavage by thioacidolysis, *Phytochemistry* 43 (1996) 1189 1194.
- [178] N. Durot, F. Gaudard, B. Kurek, The unmasking of lignin structures in wheat straw by alkali, *Phytochemistry* 63 (2003) 617 623.
- [179] H. Onnerud, Lignin structures in normal and compression wood. Evaluation by thioacidolysis using ethanethiol and methanethiol, *Holzforschung* 57 (2003) 377 384.
- [180] F.C. Lu, J. Ralph, Derivatization followed by reductive cleavage (DFRC method), a new method for lignin analysis: Protocol for analysis of DFRC monomers, *J. Agr. Food Chem.* 45 (1997) 2590 2592.
- [181] F.C. Lu, J. Ralph, DFRC method for lignin analysis. 1. New method for beta aryl ether cleavage: Lignin model studies, *J. Agr. Food Chem.* 45 (1997) 4655 4660.
- [182] F.C. Lu, J. Ralph, The DFRC method for lignin analysis. 2. Monomers from isolated lignins, *J. Agr. Food Chem.* 46 (1998) 547 552.
- [183] D.J. Yelle, J. Ralph, F.C. Lu, K.E. Hammel, Evidence for cleavage of lignin by a brown rot basidiomycete, *Environ. Microbiol.* 10 (2008) 1844 1849.
- [184] A. Guerra, L.A. Lucia, D.S. Argyropoulos, Isolation and characterization of lignins from *Eucalyptus grandis* Hill ex Maiden and *Eucalyptus globulus* Labill. by enzymatic mild acidolysis (EMAL), *Holzforschung* 62 (2008) 24 30.
- [185] A. Guerra, R. Mendonca, A. Ferraz, F.C. Lu, J. Ralph, Structural characterization of lignin during *Pinus taeda* wood treatment with *Ceripotiopsis subvermispora*, *Appl. Environ. Microb.* 70 (2004) 4073 4078.
- [186] M. Bunzel, A. Seiler, H. Steinhart, Characterization of dietary fiber lignins from fruits and vegetables using the DFRC method, *J. Agr. Food Chem.* 53 (2005) 9553 9559.
- [187] F.C. Lu, J. Ralph, Reactions of lignin model beta aryl ethers with acetyl bromide, *Holzforschung* 50 (1996) 360 364.
- [188] R.D. Hatfield, J. Grabber, J. Ralph, K. Brei, Using the acetyl bromide assay to determine lignin concentrations in herbaceous plants: Some cautionary notes, *J. Agr. Food Chem.* 47 (1999) 628 632.
- [189] I.M. Morrison, D. Stewart, Determination of lignin in the presence of ester bound substituted cinnamic acids by a modified acetyl bromide procedure, *J. Sci. Food Agr.* 69 (1995) 151 157.
- [190] F.C. Lu, J. Ralph, The DFRC method for lignin analysis. 7. Behavior of cinnamyl end groups, *J. Agr. Food Chem.* 47 (1999) 1981 1987.
- [191] J.P. Peng, F.C. Lu, J. Ralph, The DFRC method for lignin analysis. 4. Lignin dimers isolated from DFRC degraded loblolly pine wood, *J. Agr. Food Chem.* 46 (1998) 553 560.
- [192] J.P. Peng, F.C. Lu, J. Ralph, The DFRC method for lignin analysis Part 5 Isochroman lignin trimers from DFRC degraded *Pinus taeda*, *Phytochemistry* 50 (1999) 659 666.
- [193] J. Ralph, J.P. Peng, F.C. Lu, Isochroman structures in lignin: a new beta 1 pathway, *Tetrahedron Lett.* 39 (1998) 4963 4964.
- [194] F. Lu, J. Ralph, Efficient ether cleavage in lignins: the "DFRC" method as a basis for new analytical methods, in: N.G. Lewis, S. Sarkanen (Eds.), *Lignin and Lignan Biosynthesis*, American Chemical Society, Washington, DC, 1998, pp. 294 322.
- [195] J.C. Del Rio, G. Marques, J. Rencoret, A.T. Martinez, A. Gutierrez, Occurrence of naturally acetylated lignin units, *J. Agr. Food Chem.* 55 (2007) 5461 5468.
- [196] J. Ralph, F.C. Lu, The DFRC method for lignin analysis. 6. A simple modification for identifying natural acetates on lignins, *J. Agr. Food Chem.* 46 (1998) 4616 4619.
- [197] F.C. Lu, J. Ralph, Preliminary evidence for sinapyl acetate as a lignin monomer in kenaf, *Chem. Commun.* 45 (2002) 90 91.
- [198] J.C. del Rio, J. Rencoret, G. Marques, A. Gutierrez, D. Ibarra, J. I. Santos, et al., Highly acylated (acetylated and/or *p* coumaroylated) native lignins from diverse herbaceous plants, *J. Agr. Food Chem.* 56 (2008) 9525 9534.
- [199] S. Tohmura, D.S. Argyropoulos, Determination of arylglycerol beta aryl ethers and other linkages in lignins using DFRC/P 31 NMR, *J. Agr. Food Chem.* 49 (2001) 536 542.
- [200] A. Guerra, M. Norambuena, J. Freer, D.S. Argyropoulos, Determination of arylglycerol beta aryl ether linkages in enzymatic mild acidolysis lignins (EMAL): Comparison of DFRC/P 31 NMR with thioacidolysis, *J. Nat. Prod.* 71 (2008) 836 841.
- [201] C.H. Ludwig, Magnetic resonance spectra, in: K.V. Sarkanen, C.H. Ludwig (Eds.), *Lignins, Occurrence, Formation, Structure and Reactions*, Wiley Interscience, New York, 1971, pp. 299 344.
- [202] C.L. Chen, D. Robert, Characterization of Lignin by H 1 and C 13 NMR Spectroscopy, *Method. Enzymol.* 161 (1988) 137 174.
- [203] K. Lundquist, Proton (<sup>1</sup>H) NMR spectroscopy, in: S.Y. Lin, C.W. Dence (Eds.), *Methods in Lignin Chemistry*, Springer Verlag, 1992, pp. 242 249.
- [204] K. Lundquist, NMR Studies of Lignins. 5. Investigation of Non Derivatized Spruce and Birch Lignin by H 1 NMR Spectroscopy, *Acta Chem. Scand. B* 35 (1981) 497 501.
- [205] K. Lundquist, NMR studies of lignins. 4. Investigation of Spruce lignin by H 1 NMR spectroscopy, *Acta Chem. Scand. B* 34 (1980) 21 26.
- [206] H.D. Ludemann, H. Nimz, C 13 NMR Spectra of Lignins. 1. Chemical shifts of monomeric and dimeric model substances, *Makromol. Chem.* 175 (1974) 2393 2407.
- [207] H.D. Ludemann, H. Nimz, C 13 NMR spectra of lignins. 2. Beech and spruce Bjorkman lignin, *Makromol Chem.* 175 (1974) 2409 2422.
- [208] N. Terashima, R.H. Atalla, D.L. Vanderhart, Solid state NMR spectroscopy of specifically C 13 enriched lignin in wheat straw from coniferin, *Phytochemistry* 46 (1997) 863 870.
- [209] R.J. Gu, Y.M. Xie, S.Q. Zeng, H. Wu, S. Yasuda, Carbon 13 enrichment of rice stalk lignin traced by solid state C 13 NMR spectroscopy, *Chem. J. Chinese U.* 23 (2002) 1073 1076.
- [210] L.L. Landucci, Quantitative C 13 NMR Characterization of Lignin. 1. A methodology for high precision, *Holzforschung* 39 (1985) 355 359.
- [211] L.L. Landucci, Application of modern liquid state NMR to lignin characterization. 2. C 13 signal resolution and useful techniques, *Holzforschung* 45 (1991) 425 432.
- [212] T. Miyakoshi, C.L. Chen, C 13 NMR spectroscopic studies of phenylcoumaran and 1,2 Diarylpropane type lignin model compounds. 2. Substituent effects on C 13 chemical shifts of aromatic carbons, *Holzforschung* 46 (1992) 39 46.
- [213] M.G. Drumond, D.P. Veloso, S.D.S. Cota, S.A.L. Demorais, E.A. Donascimento, C.L. Chen, Biphenyl type lignin model compounds synthesis and C 13 NMR substituent chemical shift additivity rule, *Holzforschung* 46 (1992) 127 134.

- [214] J. Sipila, K. Syrjanen, Synthesis and C 13 NMR spectroscopic characterization of 6 dimeric arylglycerol Beta aryl ether model compounds representative of syringyl and *p* hydroxyphenyl structures in lignins on the aldol reaction in Beta ether preparation, *Holzforschung* 49 (1995) 325–331.
- [215] N. Terashima, R.H. Atalla, S.A. Ralph, L.L. Landucci, C. Lapierre, B. Monties, New preparations of lignin polymer models under conditions that approximate cell wall lignification. 1. Synthesis of novel lignin polymer models and their structural characterization by C 13 NMR, *Holzforschung* 49 (1995) 521–527.
- [216] L.L. Landucci, S.A. Ralph, Synthesis and C 13 NMR characterization of improved oligomeric and polymeric lignin models, *Abs. Pap. Am. Chem. Soc.* 217 (1999) 251.
- [217] L.L. Landucci, S.A. Ralph, Assessment of lignin model quality in lignin chemical shift assignments—substituent and solvent effects, *J. Wood Chem. Technol.* 17 (1997) 361–382.
- [218] D. Gagnaire, D. Robert, Polymer model of lignin (DHP) C 13 selectively labeled at benzylic positions synthesis and NMR study, *Makromol. Chem.* 178 (1977) 1477–1495.
- [219] R.L. Crawford, D.L. Crawford, [C 14] Lignin labeled lignocelluloses and C 14 labeled Milled wood lignins preparation, characterization, and uses, *Method. Enzymol.* 161 (1988) 18–31.
- [220] T.K. Kirk, G. Brunow, Synthetic C 14 labeled lignins, *Method. Enzymol.* 161 (1988) 65–73.
- [221] N. Terashima, Y. Seguchi, D. Robert, Selective C 13 enrichment of side chain carbons of guaiacyl lignin in Pine, *Holzforschung* 45 (1991) 35–39.
- [222] Y.M. Xie, N. Terashima, Selective C 13 enrichment of side chain carbons of rice stalk lignin traced by C 13 Nuclear Magnetic Resonance, *Mokuzai Gakkaishi* 39 (1993) 91–97.
- [223] Y.M. Xie, D.R. Robert, N. Terashima, Selective Carbon 13 enrichment of side chain carbons of Ginkgo lignin traced by Carbon 13 Nuclear Magnetic Resonance, *Plant Physiol. Bioch.* 32 (1994) 243–249.
- [224] Y.M. Xie, S. Yasuda, N. Terashima, Selective C 13 enrichment of side chain carbons of Oleander lignin traced by C 13 Nuclear Magnetic Resonance, *Mokuzai Gakkaishi* 40 (1994) 191–198.
- [225] Y. Xie, N. Terashima, Selective Carbon 13 enrichment of side chain carbons of Ginkgo lignin traced by C 13 Nuclear Magnetic Resonance, *Mokuzai Gakkaishi* 37 (1991) 935–941.
- [226] N. Terashima, J. Hafren, U. Westermarck, D.L. VanderHart, Nondestructive analysis of lignin structure by NMR Spectroscopy of specifically C 13 enriched lignins Part 1. Solid state study of Ginkgo wood, *Holzforschung* 56 (2002) 43–50.
- [227] R.M. Ede, G. Brunow, Application of 2 dimensional homonuclear and heteronuclear correlation NMR spectroscopy to wood lignin structure determination, *J. Org. Chem.* 57 (1992) 1477–1480.
- [228] R.M. Ede, I. Kilpelainen, Homo Nuclear and Hetero Nuclear 2d NMR techniques—unambiguous structural probes for noncyclic benzyl aryl ethers in soluble lignin samples, *Res. Chem. Intermediat.* 21 (1995) 313–328.
- [229] R.M. Ede, J. Ralph, K.M. Torr, B.S.W. Dawson, A 2D NMR investigation of the heterogeneity of distribution of diarylpropane structures in extracted *Pinus radiata* lignins, *Holzforschung* 50 (1996) 161–164.
- [230] L. Zhang, G. Gellerstedt, Observation of a novel  $\beta\beta$  structures in native lignin by high resolution 2D NMR techniques, in: Eighth European Workshop on Lignocellulosics and Pulp, Latvian State Institute of Wood Chemistry, Riga, Latvia, 2004.
- [231] I. Kilpelainen, J. Sipila, G. Brunow, K. Lundquist, R.M. Ede, Application of 2 Dimensional NMR spectroscopy to wood lignin structure determination and identification of some minor structural units of hardwood and softwood lignins, *J. Agr. Food Chem.* 42 (1994) 2790–2794.
- [232] J. Ralph, J.M. Marita, S.A. Ralph, R.D. Hatfield, F. Lu, R.M. Ede, et al., Solution state NMR of lignins, in: D.S. Argyropoulos (Ed.), *Advances in Lignocellulosics Characterization*, TAPPI Press, Atlanta, 1999, pp. 55–108.
- [233] T.M. Liitia, S.L. Maunu, B. Hortling, M. Toikka, I. Kilpelainen, Analysis of technical lignins by two- and three dimensional NMR spectroscopy, *J. Agr. Food Chem.* 51 (2003) 2136–2143.
- [234] E.A. Capanema, M.Y. Balakshin, J.F. Kadla, A comprehensive approach for quantitative lignin characterization by NMR spectroscopy, *J. Agr. Food Chem.* 52 (2004) 1850–1860.
- [235] S. Heikkinen, M.M. Toikka, P.T. Karhunen, I.A. Kilpelainen, Quantitative 2D HSQC (Q HSQC) via suppression of J dependence of polarization transfer in NMR spectroscopy: Application to wood lignin, *J. Am. Chem. Soc.* 125 (2003) 4362–4367.
- [236] L.M. Zhang, G. Gellerstedt, Quantitative 2D HSQC NMR determination of polymer structures by selecting suitable internal standard references, *Magn. Reson. Chem.* 45 (2007) 37–45.
- [237] F. Lu, J. Ralph, Non degradative dissolution and acetylation of ball milled plant cell walls; high resolution solution state NMR, *Plant J.* 35 (2003) 535–544.
- [238] D.J. Yelle, J. Ralph, C.R. Frihart, Characterization of non derivatized plant cell walls using high resolution solution state NMR spectroscopy, *Magn. Reson. Chem.* 46 (2008) 508–517.
- [239] J.M. Marita, J. Ralph, C. Lapierre, L. Jouanin, W. Boerjan, NMR characterization of lignins from transgenic poplars with suppressed caffeic acid *O* methyltransferase activity, *J. Chem. Soc. Perk. T.* 1 (2001) 2939–2945.
- [240] J.M. Marita, J. Ralph, R.D. Hatfield, D.J. Guo, F. Chen, R.A. Dixon, Structural and compositional modifications in lignin of transgenic alfalfa down regulated in caffeic acid 3 *O* methyltransferase and caffeoyl coenzyme A 3 *O* methyltransferase, *Phytochemistry* 62 (2003) 53–65.
- [241] J.M. Marita, J. Ralph, R.D. Hatfield, C. Chapple, NMR characterization of lignins in *Arabidopsis* altered in the activity of ferulate 5 hydroxylase, *Proc. Natl. Acad. Sci. U S A* 96 (1999) 12328–12332.
- [242] H. Kim, J. Ralph, T. Akiyama, Solution state 2D NMR of ball milled plant cell wall gels in DMSO  $d_6$ , *BioEnerg. Res.* 1 (2008) 56–66.
- [243] A. Bax, M.F. Summers, Proton and carbon 13 assignments from sensitivity enhanced detection of heteronuclear multiple bond connectivity by 2D multiple quantum NMR, *J. Am. Chem. Soc.* 108 (1986) 2093–2094.
- [244] N. Fukagawa, G. Meshitsuka, A. Ishizu, A 2 Dimensional NMR study of Birch milled wood lignin, *J. Wood Chem. Technol.* 11 (1991) 373–396.
- [245] N. Fukagawa, G. Meshitsuka, A. Ishizu, 2d NMR study of residual lignin in Beech Kraft pulp combined with selective cleavage with pivaloyl iodide, *J. Wood Chem. Technol.* 12 (1992) 425–445.
- [246] S. Quideau, J. Ralph, A biomimetic route to lignin model compounds via silver (I) oxide oxidation. 2. NMR characterization of noncyclic benzyl aryl ether trimers and tetramers, *Holzforschung* 48 (1994) 124–132.
- [247] S. Quideau, J. Ralph, Lignin ferulate cross links in grasses. 4. Incorporation of 5,5 coupled dehydrodiferulate into synthetic lignin, *J. Chem. Soc. Perk. T.* 1 (1997) 2351–2358.
- [248] J. Ralph, R.F. Helm, S. Quideau, R.D. Hatfield, Lignin feruloyl ester cross links in grasses. 1. incorporation of feruloyl esters into

- coniferyl alcohol dehydrogenation polymers, *J. Chem. Soc. Perk. T.* 1 (1992) 2961–2969.
- [249] J. Ralph, An unusual lignin from kenaf, *J. Nat. Prod.* 59 (1996) 341–342.
- [250] M.M. Campbell, R.R. Sederoff, Variation in lignin content and composition Mechanism of control and implications for the genetic improvement of plants, *Plant Physiol.* 110 (1996) 3–13.
- [251] M. Bunzel, J. Ralph, P. Bruning, H. Steinhart, Structural identification of dehydrotriferulic and dehydrotetraferulic acids isolated from insoluble maize bran fiber, *J. Agr. Food Chem.* 54 (2006) 6409–6418.
- [252] J.H. Grabber, J. Ralph, R.D. Hatfield, Model studies of ferulate coniferyl alcohol cross product formation in primary maize walls: Implications for lignification in grasses, *J. Agr. Food Chem.* 50 (2002) 6008–6016.
- [253] T.B.T. Lam, K. Kadota, K. Iiyama, Bonding of hydroxycinnamic acids to lignin: ferulic and *p* coumaric acids are predominantly linked at the benzyl position of lignin, not the beta position, in grass cell walls, *Phytochemistry* 57 (2001) 987–992.
- [254] J. Ralph, R.D. Hatfield, J.H. Grabber, H.G. Jung, S. Quideau, R.F. Helm, Cell wall cross linking in grasses by ferulates and diferulates, in: N.G. Lewis, S. Sarkanen (Eds.), *Lignin and Lignan Biosynthesis*, American Chemical Society, Washington, DC, 1998, pp. 209–236.
- [255] A. Scalbert, B. Monties, C. Rolando, A. Sierraescudero, Formation of ether linkage between phenolic acids and Gramineae lignin: A possible mechanism involving quinone methides, *Holzforschung* 40 (1986) 191–195.
- [256] T. Aoyagi, S. Hosoya, J. Nakano, New reaction site in lignins during the oxygen alkali treatment, *Mokuzai Gakkaishi* 21 (1975) 532–534.
- [257] Y. Kato, D.J. Nevins, Isolation and identification of O (5 O feruloyl  $\alpha$  L arabinofuranosyl) (1 3) O  $\beta$  D xylopyranosyl (1 4) D xylose as a component of Zea shoot cell walls, *Carbohydr. Res.* 137 (1985) 139–150.
- [258] F.C. Lu, J. Ralph, A.P. Zhang, M. Bunzel, A novel approach to identify the 8 5 cross coupled dimer between coniferyl alcohol and ferulate in plant cell walls, in: *Ferulate '08*, Minneapolis/St. Paul, 2008.
- [259] J.H. Grabber, J. Ralph, R.D. Hatfield, Cross linking of maize walls by ferulate dimerization and incorporation into lignin, *J. Agr. Food Chem.* 48 (2000) 6106–6113.
- [260] J.H. Grabber, J. Ralph, R.D. Hatfield, S. Quideau, T. Kuster, A.N. Pell, Dehydrogenation polymer cell wall complexes as a model for lignified grass walls, *J. Agr. Food Chem.* 44 (1996) 1453–1459.
- [261] J.H. Grabber, J. Ralph, C. Lapierre, Y. Barriere, Genetic and molecular basis of grass cell wall degradability. I. Lignin cell wall matrix interactions, *C. R. Biologies* 327 (2004) 455–465.
- [262] S.M. Carnachan, P.J. Harris, Ferulic acid is bound to the primary cell walls of all gymnosperm families, *Biochem. Syst. Ecol.* 28 (2000) 865–879.
- [263] D.C.C. Smith, *p* Hydroxybenzoates groups in the lignin of Aspen (*Populus tremula*), *J. Chem. Soc.* (1955) part III, 2347–2351.
- [264] J. Nakano, A. Ishizu, N. Migita, Studies on lignin. XXXII. Ester groups of lignin, *Tappi* 44 (1961) 30–32.
- [265] L.L. Landucci, G.C. Deka, D.N. Roy, A C 13 NMR study of milled wood lignins from hybrid salix clones, *Holzforschung* 46 (1992) 505–511.
- [266] R.C. Sun, J.M. Fang, J. Tomkinson, Fractional isolation and structural characterization of lignins from oil palm trunk and empty fruit bunch fibers, *J. Wood Chem. Technol.* 19 (1999) 335–356.
- [267] H. Meyermans, K. Morreel, C. Lapierre, B. Pollet, A. De Bruyn, R. Busson, et al., Modifications in lignin and accumulation of phenolic glucosides in poplar xylem upon down regulation of caffeoyl coenzyme A *O* methyltransferase, an enzyme involved in lignin biosynthesis, *J. Biol. Chem.* 275 (2000) 36899–36909.
- [268] S.M. Li, K. Lundquist, Analysis of hydroxyl groups in lignins by H 1 NMR spectrometry, *Nord. Pulp Pap. Res. J.* 16 (2001) 63–67.
- [269] M. Shimada, T. Fukuzuka, T. Higuchi, Ester linkages of *p* coumaric acid in bamboo and grass lignins, *Tappi* 54 (1971) 72–77.
- [270] R.D. Hartley, *p* Coumaric and ferulic acid components of cell walls of ryegrass and their relationships with lignin and digestibility, *J. Sci. Food Agr.* 23 (1972) 1347–1354.
- [271] P.J. Harris, R.D. Hartley, Phenolic constituents of the cell walls of monocotyledons, *Biochem. Syst. Ecol.* 8 (1980) 153–160.
- [272] T.A. Morrison, H.G. Jung, D.R. Buxton, R.D. Hatfield, Cell wall composition of maize internodes of varying maturity, *Crop Sci.* 38 (1998) 455–460.
- [273] M.A.B. Vailhe, G.J. Provan, L. Scobbie, A. Chesson, M.P. Maillot, A. Cornu, et al., Effect of phenolic structures on the degradability of cell walls isolated from newly extended apical internode of tall fescue (*Festuca arundinacea* Schreb.), *J. Agr. Food Chem.* 48 (2000) 618–623.
- [274] R. Hatfield, J.M. Marita, K. Frost, J.H. Grabber, J. Ralph, F.C. Lu, et al., Grass lignin acylation: *p* coumaroyl transferase activity and cell characteristics of C<sub>3</sub> and C<sub>4</sub> grasses, *Planta* 229 (2009) 1253–1267.
- [275] A. Scalbert, B. Monties, E. Guittet, J.Y. Lallemand, Comparison of wheat straw lignin preparations. 1. Chemical and spectroscopic characterizations, *Holzforschung* 40 (1986) 119–127.
- [276] K. Iiyama, T.B.T. Lam, Lignin in wheat internodes. 1. The reactivities of lignin units during alkaline nitrobenzene oxidation, *J. Sci. Food Agr.* 51 (1990) 481–491.
- [277] J. Ralph, P.F. Schatz, F.C. Lu, H. Kim, T. Akiyama, S.F. Nelsen, Quinone methides in lignification, in: S.E. Rokita (Ed.), *Quinone Methides*, John Wiley, Hoboken, NJ, 2008, pp. 385–420.
- [278] R. Hatfield, J. Ralph, J.H. Grabber, A potential role for sinapyl *p* coumarate as a radical transfer mechanism in grass lignin formation, *Planta* 228 (2008) 919–928.
- [279] U. Takahama, T. Oniki, Effects of ascorbate on the oxidation of derivatives of hydroxycinnamic acid and the mechanism of oxidation of sinapic acid by cell wall bound peroxidases, *Plant Cell Physiol.* 35 (1994) 593–600.
- [280] U. Takahama, Oxidation of hydroxycinnamic acid and hydroxy cinnamyl alcohol derivatives by Laccase and peroxidase interactions among *p* Hydroxyphenyl, guaiacyl and syringyl groups during the oxidation reactions, *Physiol. Plantarum* 93 (1995) 61–68.
- [281] U. Takahama, T. Oniki, H. Shimokawa, A possible mechanism for the oxidation of sinapyl alcohol by peroxidase dependent reactions in the apoplast: Enhancement of the oxidation by hydroxycinnamic acids and components of the apoplast, *Plant Cell Physiol.* 37 (1996) 499–504.
- [282] J.H. Grabber, F.C. Lu, Formation of syringyl rich lignins in maize as influenced by feruloylated xylans and *p* coumaroylated monolignols, *Planta* 226 (2007) 741–751.
- [283] A. Björkman, Lignin and lignin carbohydrate complexes Extraction from wood meal with neutral solvents, *Ind. Eng. Chem.* 49 (1957) 1395–1398.
- [284] Y.Z. Lai, K.V. Sarkanen, Isolation and structural studies, in: K.V. Sarkanen, C.H. Ludwig (Eds.), *Lignins, Occurrence, Formation, Structure and Reactions*, Wiley Interscience, New York, 1971, pp. 165–240.

- [285] J.W.T. Merewether, A lignin carbohydrate complex in wood, *Holzforschung* 11 (1957) 65 80.
- [286] J. Azuma, Analysis of lignin carbohydrate complexes of plant cell walls, in: H.F. Linskens, J.F. Jackson (Eds.), *Morden Methods of Plant Analysis*, Springer, New York, London, Paris, Tokyo, 1989, pp. 100 126.
- [287] H.H. Brownell, Stability of lignin carbohydrate complex, *Tappi* 54 (1971) 66 71.
- [288] I.M. Morrison, Isolation and analysis of lignin carbohydrate complexes from *Iolium multiflorum*, *Phytochemistry* 12 (1973) 2979 2984.
- [289] O. Eriksson, B.O. Lindgren, About linkage between lignin and hemicelluloses in wood, *Sven. Papperstid.* 80 (1977) 59 63.
- [290] J.L. Minor, Chemical linkage of pine polysaccharides to lignin, *J. Wood Chem. Technol.* 2 (1982) 1 16.
- [291] J.R. Obst, Frequency and alkali resistance of lignin carbohydrate bonds in wood, *Tappi* 65 (1982) 109 112.
- [292] C.W. Ford, Comparative structural studies of lignin carbohydrate complexes from *Digitaria Decumbens* (Pangola Grass) before and after chlorite delignification, *Carbohyd. Res.* 147 (1986) 101 117.
- [293] B. Mouttet, A. Robert, Lignin polysaccharide complexes in *Sequoia Sempervirens* stems cultivated invitro, *Holzforschung* 32 (1978) 173 177.
- [294] G. Wegener, Contribution to characterization of sodium chlorite delignification of Spruce wood. 3. Lignin polysaccharide complexes in chlorite liquors, *Papier* 30 (1976) 177 185.
- [295] A.J. Glinski, G.A. Nicholls, The peroxyacetic acid delignification of white birch and new evidence for lignin carbohydrate bonds, *Pap. Puu Pap. Tim.* 59 (1977) 745 749.
- [296] W.S. Linnell, N.S. Thompson, H.A. Swenson, Structure of black Spruce glucomannan. 2. Lignin glucomannan relationships, *Tappi* 49 (1966) 491 493.
- [297] K.P. Kringstad, C.W. Cheng, Studies on lignin hemicellulose complexes, *Tappi* 52 (1969) 2382 2385.
- [298] J.L. Minor, Chemical linkage of polysaccharides to residual lignin in Loblolly Pine Kraft pulps, *J. Wood Chem. Technol.* 6 (1986) 185 201.
- [299] T. Iversen, U. Westmark, Lignin carbohydrate bonds in Pine lignins dissolved during Kraft pulping, *Cell. Chem. Technol.* 19 (1985) 531 536.
- [300] B. Kosikova, J. Polcin, Dandarov. M, A new method for isolation of lignin carbohydrate complex, *Holzforschung* 23 (1969) 37 43.
- [301] B. Kosikova, J. Polcin, Isolation of lignin from Spruce by acidolysis in dioxane, *Wood Sci. Technol.* 7 (1973) 308 316.
- [302] J.P. Joseleau, R. Kesraoui, Glycosidic bonds between lignin and carbohydrates, *Holzforschung* 40 (1986) 163 168.
- [303] T. Fukuda, M. Kohmoto, Studies on tissue culture of tree cambium. 10. Lignin carbohydrate complex in suspension cultured cells of *Robinia Pseudoacacia*, *Mokuzai Gakkaishi* 32 (1986) 827 832.
- [304] F.W. Whitmore, Lignin carbohydrate complex formed in isolated cell walls of Callus, *Phytochemistry* 17 (1978) 421 425.
- [305] J.I. Azuma, K. Tetsuo, Lignin carbohydrate complexes from various sources, *Method. Enzymol.* 161 (1988) 12 18.
- [306] M.Y. Balakshin, E.A. Capanema, H.M. Chang, MWL fraction with a high concentration of lignin carbohydrate linkages: Isolation and 2D NMR spectroscopic analysis, *Holzforschung* 61 (2007) 1 7.
- [307] W. Zimmermann, A. Paterson, P. Broda, Conventional and High Performance Size Exclusion chromatography of graminaceous lignin carbohydrate complexes, *Method. Enzymol.* 161 (1988) 191 199.
- [308] G. Wallace, W.R. Russell, J.A. Lomax, M.C. Jarvis, C. Lapierre, A. Chesson, Extraction of phenolic carbohydrate complexes from graminaceous cell walls, *Carbohyd. Res.* 272 (1995) 41 53.
- [309] J. Ohnishi, T. Watanabe, T. Koshijima, Synthesis of dehydrogenation polymer polyose complexes by peroxidase, *Phytochemistry* 31 (1992) 1185 1190.
- [310] A. Barakat, B. Chabbert, B. Cathala, Effect of reaction media concentration on the solubility and the chemical structure of lignin model compounds, *Phytochemistry* 68 (2007) 2118 2125.
- [311] A. Barakat, H. Winter, C. Rondeau Mouro, B. Saake, B. Chabbert, B. Cathala, Studies of xylan interactions and cross linking to synthetic lignins formed by bulk and end wise polymerization: a model study of lignin carbohydrate complex formation, *Planta* 226 (2007) 267 281.
- [312] R. Whetten, R. Sederoff, Lignin biosynthesis, *Plant Cell* 7 (1995) 1001 1013.
- [313] S. Lin, Lignin utilization: potential and challenge, in: D.A. Tillman, E.C. Jahn (Eds.), *Progress in Biomass Conversion*, Academic Press, Inc, 1983, pp. 31 78.
- [314] A. Pizzi, Recent developments in eco efficient bio based adhesives for wood bonding: opportunities and issues, *J. Adhes. Sci. Technol.* 20 (2006) 829 846.
- [315] Y. Liu, K.C. Li, Development and characterization of adhesives from soy protein for bonding wood, *Int. J. Adhes. Adhes.* 27 (2007) 59 67.
- [316] A. Despres, A. Pizzi, C. Vu, H. Pasch, Formaldehyde free Aminoresin wood adhesives based on dimethoxyethanal, *J. Appl. Polym. Sci.* 110 (2008) 3908 3916.
- [317] N.G. Lewis, T.R. Lantzy, S.J. Branham, Lignin in adhesives: Introduction and historical perspective, in: R.W. Hemingway, A.H. Conner (Eds.), *Adhesives from Renewable Resources*, Oxford University Press, 1989, pp. 13 26.
- [318] H. Nimz, Lignin based wood adhesives, in: A. Pizzi (Ed.), *Wood Adhesives: Chemistry and Technology*, Marcel Dekker, Inc., New York, 1983, pp. 247 288.
- [319] K.G. Forss, A. Fuhrmann, Finnish plywood, particleboard, and fiberboard made with a lignin base adhesive, *Forest Prod. J.* 29 (1979) 39 43.
- [320] K.C. Shen, L. Calve, Improving by fractionation ammonium based spent sulfite liquor for waferboard binder, *Adhes. Age* 23 (1980) 25 29.
- [321] R.C. Gupta, S.P. Singh, S. Jolly, Phenol lignin formaldehyde adhesives for plywood, *Holzforschung Und Holzverwertung* 30 (1978) 109 112.
- [322] W. Peng, A.O. Barry, B. Riedl, Characterization of methylolated lignin by <sup>1</sup>H NMR and <sup>13</sup>C NMR, *J. Wood Chem. Technol.* 12 (1992) 299 312.
- [323] M.V. Alonso, M. Oliet, F. Rodriguez, J. Garcia, M.A. Gilarranz, J.J. Rodriguez, Modification of ammonium lignosulfonate by phenolation for use in phenolic resins, *Bioresource Technol.* 96 (2005) 1013 1018.
- [324] R.C. Gupta, V.K. Sehgal, Effect of viscosity and molecular weight of lignin phenol formaldehyde resin on the glue adhesion strength of plywood, *Holzforschung Und Holzverwertung* 31 (1979) 7 9.
- [325] M.V. Alonso, J.J. Rodriguez, M. Oliet, F. Rodriguez, J. Garcia, M.A. Gilarranz, Characterization and structural modification of ammonic lignosulfonate by methylation, *J. Appl. Polym. Sci.* 82 (2001) 2661 2668.
- [326] H. Elsaied, A.M. Nada, A.A. Ibrahim, M.A. Yousef, Waste Liquors from Cellulosic Industries. 3. Lignin from Soda Spent Liquor as a



- Component in Phenol Formaldehyde Resin, *Angew. Makromol. Chem.* 122 (1984) 169–181.
- [327] A.M.A. Nada, H. Elsaied, A.A. Ibrahim, M.A. Yousef, Waste liquors from cellulosic industries. 4. Lignin as a component in phenol formaldehyde resol resin, *J. Appl. Polym. Sci.* 33 (1987) 2915–2924.
- [328] R.E. Ysbrandy, R.D. Sanderson, G.F.R. Gerischer, Adhesives from autohydrolysis bagasse lignin, a Renewable Resource. 1. The physical properties of laminates made with phenolated lignin novolacs, *Holzforschung* 46 (1992) 249–252.
- [329] G. Vazquez, J. Gonzalez, S. Freire, G. Antorrena, Effect of chemical modification of lignin on the gluebond performance of lignin phenolic resins, *Bioresource Technol.* 60 (1997) 191–198.
- [330] L.R. Calve, J.A. Shields, L. Blanchette, J.M.J. Frechet, A practical lignin based adhesive for waferboard/Osb, *Forest Prod. J.* 38 (1988) 15–20.
- [331] J.J. Lindberg, T.A. Kuusela, K. Levon, Specialty polymers from lignin, in: W.G. Glasser, S. Sarkanen (Eds.), *Lignin: Properties and Materials*, American Chemical Society, 1989, pp. 190–204.
- [332] G. Vazquez, G. Antorrena, J. Gonzalez, J. Mayor, Lignin phenol formaldehyde adhesives for exterior grade plywoods, *Bioresource Technol.* 51 (1995) 187–192.
- [333] R.S.J. Piccolo, F. Santos, E. Frollini, Sugar cane bagasse lignin in resol type resin: alternative application for lignin phenol formaldehyde resins, *J. Macromol. Sci. Pure A34* (1997) 153–164.
- [334] T. Malutan, R. Nicu, V.I. Popa, Contribution to the study of hydroxymethylation reaction of alkali lignin, *Bioresources* 3 (2008) 13–20.
- [335] G.H. Vanderklashorst, F.A. Cameron, A. Pizzi, Lignin based cold setting wood adhesives structural fingerjoints and glulam, *Holz Roh. Werkst.* 43 (1985) 477–481.
- [336] R.E. Ysbrandy, R.D. Sanderson, G.F.R. Gerischer, Adhesives from autohydrolysis bagasse lignin, a renewable resource. 2. Dsc thermal analysis of novolac resins, *Holzforschung* 46 (1992) 253–256.
- [337] G.Y. Liu, X.Q. Qiu, D.S. Xing, D.J. Yang, Phenolation modification of wheat straw soda lignin and its utilization in lignin based phenolic formaldehyde resins, in: B.H. He, S.Y. Fu, F.G. Chen (Eds.), *3rd International Symposium on Emerging Technologies of Pulp and Papermaking*, South China Univ Technology Press, Guangzhou, 2006, pp. 933–938.
- [338] K.C. Li, X.L. Geng, Investigation of formaldehyde free wood adhesives from kraft lignin and a polyaminoamide epichlorohydrin resin, *J. Adhes. Sci. Technol.* 18 (2004) 427–439.
- [339] X.L. Geng, K.C. Li, Investigation of wood adhesives from kraft lignin and polyethylenimine, *J. Adhes. Sci. Technol.* 20 (2006) 847–858.
- [340] Y. Liu, K. Li, Preparation and characterization of demethylated lignin polyethylenimine adhesives, *J. Adhesion* 82 (2006) 593–605.
- [341] M. Balat, H. Balat, C. Oz, Progress in bioethanol processing, *Prog. Energ. Combust.* 34 (2008) 551–573.
- [342] M. Woodson, C.J. Jablonowski, An economic assessment of traditional and cellulosic ethanol technologies, *Energ. Source. B* 3 (2008) 372–383.
- [343] M.A. Ratcliff, D.K. Johnson, F.L. Posey, H.L. Chum, Hydrodeoxygenation of lignins and model compounds, *Appl. Biochem. Biotech.* 17 (1988) 151–160.
- [344] J. Piskorz, P. Majerski, D. Radlein, D.S. Scott, Conversion of lignins to hydrocarbon fuels, *Energ. Fuel.* 3 (1989) 723–726.
- [345] A. Oasmaa, R. Alen, D. Meier, Catalytic hydrotreatment of some technical lignins, *Bioresource Technol.* 45 (1993) 189–194.
- [346] A. Oasmaa, A. Johansson, Catalytic hydrotreating of lignin with water soluble molybdenum catalyst, *Energ. Fuel.* 7 (1993) 426–429.
- [347] M.M. Hepditch, R.W. Thring, Degradation of solvolysis lignin using Lewis acid catalysts, *Can. J. Chem. Eng.* 78 (2000) 226–231.
- [348] R.W. Thring, S.P.R. Katikaneni, N.N. Bakhshi, The production of gasoline range hydrocarbons from Alcell (R) lignin using HZSM 5 catalyst, *Fuel Process. Technol.* 62 (2000) 17–30.
- [349] M. Kleinert, T. Barth, Towards a lignin-cellulosic biorefinery: Direct one step conversion of lignin to hydrogen enriched biofuel, *Energ. Fuel.* 22 (2008) 1371–1379.
- [350] G. Gellerstedt, J.B. Li, I. Eide, M. Kleinert, T. Barth, Chemical structures present in biofuel obtained from lignin, *Energ. Fuel.* 22 (2008) 4240–4244.
- [351] J. Shabtai, W. Zmierzak, E. Chornet, Process for conversion of lignin to reformulated hydrocarbon gasoline, in US patent 5,959,167, (1998).
- [352] G.S. Macala, T.D. Matson, C.L. Johnson, R.S. Lewis, A.V. Iretskii, P.C. Ford, Hydrogen transfer from supercritical methanol over a solid base catalyst: A model for lignin depolymerization, *Chem SusChem* 2 (2009) 215–217.
- [353] J. Shabtai, W. Zmierzak, E. Chornet, Process for conversion of lignin to reformulated, partially oxygenated gasoline, in US patent 6,172,272, (2001).
- [354] N. Yan, C. Zhao, P. Dyson, C. Wang, L. Liu, Y. Kou, Selective degradation of wood lignin over noble metal catalysts in a two step process, *ChemSusChem* 1 (2008) 626–629.



# Chemical Modification of Straw as Novel Materials for Industries

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## 7.1 CHEMICAL MODIFICATION OF CEREAL STRAWS AS NATURAL SORBENTS IN OIL SPILL CLEANUP

### 7.1.1 Hazard of Oil Spill

Oil is one of the most important energy and raw material source for the synthesis of synthetic polymers and chemicals world wide [1]. As long as oil is explored, transported, stored, and used, there will be the risk of a spillage. In recent years, tremendous increases of accidental and intentional oil spillage have occurred during production, transportation, and refining [2]. Oil pollution, particularly of sea and navigable waters, has stimulated more public concern than other waste or spilt materials. Oil pollution of the sea has steadily increased with the increase in oil consumption. The total annual influx of petroleum hydrocarbons is about 10 million metric tons. The bulk of this influx is due to the transportation-related activities spill from tanker loading and unloading operations, pipeline rupture, which may be because of industrial waste as leakage from engines, incorrect operation of valves, and discharge of oily wastes. Oil pollution of the shore, in addition to the reduction of amenity, also affects marine, shore life, and vegetation [3]. Crude oil consists of different hydrocarbons that range from light gas to heavy solids. When oil is spilled on water, the physical and chemical properties of the oil change progressively, i.e., these physicochemical changes enhance oil dissolution in seawater [4]. This process is referred to as weathering, which includes evaporation, dissolution, dispersion, photochemical oxidation, microbial degradation, adsorption onto suspended materials, agglomeration, and so on. [5]. Spilled oil has an undesirable taste and odor, causes severe environmental damage on waterfowl, material life, and affects tourism and economy [6].

### 7.1.2 Methods for Controlling Oil Spills

Oil spills are a global concern due to the environmental and economical impact. Spilled oil causes immense environmental damage unless it is removed as quickly as possible. Thus, various commercial systems have been developed to control these spills, e.g., removing oil from contaminated areas by the use of booms, dispersants, skimmers, oil water separator, or different kinds of sorbent materials [7]. However, some of the main limitations of these techniques are their high cost and inefficient trace level adsorption [8]. One of the most economical and efficient means for the removal of spilled oil from either land or sea is the use of sorbents [9]. Because the sorbent oil mixture has decidedly different physical properties compared with fluid, this will aid in retrieval operations. Another benefit of using a sorbent is its capability to capture and retain oil for retrieval at a later time. This may be a major advantage due to the rapid initial rate of spreading of oil slicks on water. Additional advantages of a properly chosen sorbent are selectivity, effectiveness, and general application. Ideally, a sorbent should pick up at least 92% of the oil from the water surface. Finally, sorbents can function with oil slicks of varying thicknesses and viscosities. Such a property is essential for the design of general systems that can handle a majority of spill situations [10].

### 7.1.3 Classification of Oil Sorbents

Sorbents are materials that absorb liquids. They can be used to recover oil through the mechanisms of absorption, adsorption, or both. Absorbents allow oil to penetrate into pore spaces in the material they are made of, whereas adsorbents attract oil to their surfaces but do not allow it to penetrate into the material. To be useful in combating oil spills, sorbents need to be both

oleophilic and hydrophobic (water repellent). Several studies of different natural, synthetic, and mineral sorbents have been conducted [11–13]. A loose fibrous material, which is oleophilic, hydrophobic, and floatable on water for selective absorption of hydrocarbons, was produced [14]. Although sorbents may be used as the sole cleanup method in small spills, they are most often used to remove final traces of oil or in areas that cannot be reached by skimmers. Once sorbents have been used to recover oil, they must be removed from the water and properly disposed of on land or cleaned for reuse. Any oil that is removed from sorbent materials should be properly disposed of or recycled.

The following characteristics must be considered when choosing sorbents for cleaning up spills [15]:

1. **Rate of absorption or adsorption:** The rate of absorption varies with the thickness of the oil. Light oils are absorbed more quickly than heavy ones.
2. **Oil retention:** The weight of recovered oil can cause a sorbent structure to sag and deform. When it is lifted out of the water, it can release oil that is trapped in its pores. During recovery of absorbent materials, lighter, less viscous oil is lost through the pores more easily than heavier, more viscous oil.
3. **Ease of application:** Sorbents may be applied to spills manually or mechanically, using blowers or fans. Many natural organic sorbents that exist as loose materials, such as clay and vermiculite, are dusty, difficult to apply in windy conditions, and potentially hazardous if inhaled.

Sorbents can be divided into three basic categories: natural inorganic, natural organic, and synthetic products.

*Natural inorganic sorbents* include clay, perlite, vermiculite, glass, wool, sand, and volcanic ash. They can absorb from 4 to 20 times their weight in oil. These materials do not show adequate buoyancy retention, and their oil sorption capacity is generally low [16].

*Synthetic sorbents*, such as polypropylene and polyurethane, are the most commonly used commercial sorbents in oil spill cleanup because of their oleophilic and hydrophobic characteristics [17]. Most synthetic sorbents can absorb as much as 70 times their weight in oil, and some types can be cleaned and reused several times. They have good hydrophobic and oleophilic properties, but their nonbiodegradability is a major disadvantage [16, 18]. A biodegradable material with excellent absorption properties would be advantageous in this respect. Thus, a number of natural biodegradable sorbents have been studied for use in oil spill cleanup, e.g., cotton [9, 11, 19], wool [20], and bark [21], which can be an excellent oil sorbent because of their hydrophobic and oleophilic characteristics.

*Natural organic sorbents* include peat moss, straw, hay, sawdust, ground corncobs, feathers, and other carbon-based products [15]. The usage of waste materials or by-products from agriculture as inexpensive alternative materials for environmental remediation had been a subject of interest

among many researchers [22, 23]. They are relatively inexpensive and usually readily available. Organic sorbents can absorb from 3 to 15 times their weight in oil, but they do have some disadvantages. Some organic sorbents tend to absorb water as well as oil, causing them to sink. Many organic sorbents are loose particles, such as sawdust, and are difficult to collect after they are spread on the water [15]. Organic sorbents, such as straw, corn cob, rice hulls, peat moss, feathers, wood chips, and wool, have recently been used more frequently because of the following factors: 1) biodegradability, 2) renewable resources, 3) waste recycling of life cycle extension, 4) lower cost per unit, 5) lower impact on ecosystem if released or lost during major spill cleanup operations, and 6) public perception that the products are environment friendly [24].

## 7.1.4 Principles of Sorbency

### 7.1.4.1 Absorption

A good sorbent is sponge-like, with holes in the surface that draw oil into the interior pores. The percentage of pore space, termed porosity, is an important property of sorbents because materials with high porosity can absorb large volume of oil in a bed with given dimensions [24]. Fibrous beds also have the advantage of being squeezable, thereby permitting recovery of oil and reuse of the sorbent. When fibrous beds are subjected to compressive loads, the porosity is decreased. The amount of oil that can be extracted from a sorbent bed depends on the porosity, the time of contact, and the compressive pressure applied by a roll, diaphragm, or piston. Removing oil by squeezing has its analog in solid/liquid separation when fluid is extracted from cakes after filtration is complete.

### 7.1.4.2 Adsorption

Adsorbents are natural or synthetic materials of microcrystalline structure, whose internal pore surfaces are accessible for selective combinations of solid and solute. Usually, the attractive forces are weaker and less specific than those of the chemical bonds. Hence, adsorption is analogous to a condensation of gas molecules or to crystallization of a liquid. Its selective action is more pronounced in a monomolecular layer next to the solid surface, but at times, selectivity may persist to a height of three or four molecules. This combining effect is known as physical adsorption. The adsorption capacity of solute tends to increase with the increase in fluid-phase concentration of the solute. At higher temperatures, adsorption may occur through a true reaction or chemical bonding; the process is then termed chemisorption [24].

## 7.1.5 Influence of Sorptive Capacity

Several important factors that influence sorptive capacity of sorbents are density, porosity, selectivity, and retention [25].

### 7.1.5.1 Density

There are two types of density relative to absorption: true density and bulk density. True density is a measure of solids only, regardless of any internal voids or interstitial areas, and once determined, it can be considered constant for a given material. To determine true density, both mass and volume must be known. Volume can be determined by submerging a known mass of material into a container of known volume of a wetting liquid. The liquid must be allowed to fully penetrate the material and displace any entrapped gasses. It may be necessary to augment the penetration with vacuum or agitation. True density can then be determined by the following formula:

$$D_s = D_L \frac{M_s}{M_1 + M_s - M_2},$$

where  $D_s$  is the density of the solid,  $D_L$  is the density of the wetting liquid,  $M_s$  is the mass of the solid,  $M_1$  is the mass of liquid required to fill the measuring container without fiber, and  $M_2$  is the mass of liquid required to fill the measuring container with  $M_s$  in the container.

Bulk density is a measure of density including solids, pores, and interstices and may vary depending on compaction. It is a simple measure of mass/unit volume. For fibrous materials, bulk density varies widely. Incompacted bulk densities can approach true density.

### 7.1.5.2 Porosity

Porosity, or void volume, is a measure of how much volume is available in a system for absorption. Like bulk density, porosity may vary depending on compaction. Porosity can be expressed as follows:

$$P_R = \frac{V - V_s}{V},$$

where  $P_R$  is the porosity (%),  $V$  is the total volume of the system, and  $V_s$  is the volume of the solid.

At first glance, porosity may appear to be an indicator of absorptive capacity. In fact, it is a measure of a system's capacity only under ideal conditions. In practice, a sorbent's capacity is often less than its porosity. Fibrous materials often have porosities of 90–95%, whereas granular materials' porosity is often less than 40%. Porosity is also often expressed as void ratio,  $P$ . Void ratio is the ratio of the void volume to the solid volume and is expressed as follows:

$$P = \frac{V - V_s}{V_s}$$

### 7.1.5.3 Selectivity

Selectivity is the capability of a sorptive material to preferentially absorb one material over another. For instance, most agro-based materials will, to varying degrees, selectively absorb oil over water. This makes these

materials attractive sorbents in oil spills caused by tanker and offshore oil rig leaks. The degree of selectivity is influenced by the sorbent's pore size, wettability, and capillary pressure. History of the sorbent is also important in selectivity. For instance, in the case of oil spills, the sorbent's capability to preferentially absorb oil over sea water is affected by whether the sorbent was exposed to the oil first or the water.

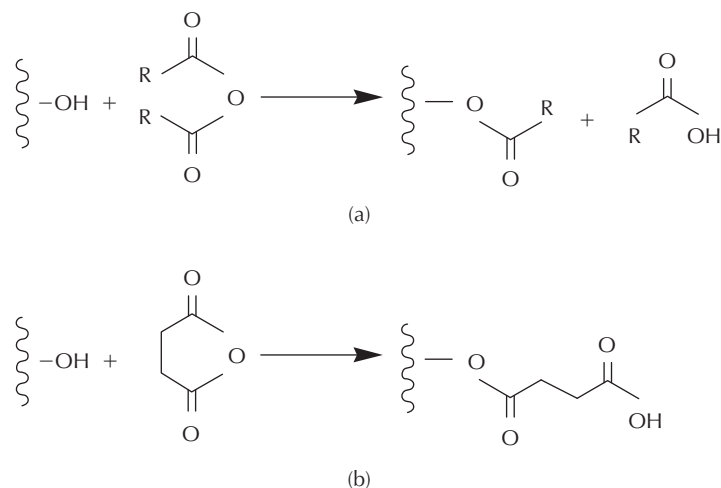
### 7.1.5.4 Retention

Retention is the capability of a saturated sorptive material to retain fluid when conditions are conducive to drainage. Retention is important because in practice, sorbents are often used in one location and transported to another for disposal or fluid removal. A sorbent with high degree of retention will be able to transport more fluid. Retention levels are based largely on conditions related to the capillary action. Generally, at equilibrium, a saturated sorbent holds more fluid after drainage than an unsaturated sorbent takes in. This is because the capillary nature of sorbent systems, especially those made of irregular agro-based materials, is not regular. During drainage, fluid stops when a neck in the capillary system is small enough so that the pressure difference is enough to keep the liquid column in place. During absorption, the liquid only enters the sorbent until it reaches a wide area where the pressure drop is insufficient to take it any further into the sorbent system. Because agro-fibers vary widely, the equilibrium point varies throughout the sorbent system.

In a word, the preferable sorbent materials are those which, besides being inexpensive and readily available, demonstrate fast oil sorption rate, high oil sorption capacity (oleophilicity or lipophilicity), low water pickup, high oil retention capacity during transfer, high recovery of the absorbed oil with simple methods, good reusability, high buoyancy, excellent physical and chemical resistances against deformation, photodegradation, and chemical attacks [26].

## 7.1.6 Chemical Modification of Cereal Straws as Oil Sorbents

Increasing environmental concern, especially after several hazardous incidents in the past decades when large quantities of oil were spilled into the sea, renewed the interest for natural fibers [20]. The chemical modification of wood with anhydride reagents has been the subject of research for many decades [27–30]. Acetylation has been the most widely used and successful chemical modification and is a single-site reaction that replaces a hydroxyl group with an acetyl group (Fig. 7.1.1) [31]. Acetyl groups are more hydrophobic than hydroxyl groups; therefore, replacing some of the hydroxyl groups with acetyl groups



**FIGURE 7.1.1** The reaction formula of wood and linear anhydride (a) and ring like anhydride (b) [31].

reduces the hydrophilic property of the cell wall polymers [32]. The acetyl group is also larger than the hydroxyl group; therefore, the material undergoes permanent expansion [33]. In general, the reaction leads to an increased content of acetyl groups in the wood material, to approximately 20 wt% compared with 1–2 wt% for unmodified wood. The introduction of new acetyl groups in wood polymers results in a certain degree of bulking of the wood cell walls. This, in combination with the decreased ability to attract water molecules, leads to highly improved dimensional stability of acetylated wood material [34]. On the other hand, because of the chemical modification of whole wood, effective penetration of the reagent to the bulk of the material becomes difficult, and subsequently the unreacted chemicals and by-products need to be removed. Because of these problems, many studies have concentrated upon the modification of wood fiber or chips, but the economics of such a process is in some doubt [27]. In addition, with decreasing wood resources, work has increased regarding the use of nonwood products such as cereal straws, like raw materials for industrial use.

Application of agro-industrial residues in industries on the one hand provides alternative substrates and on the other hand helps in solving pollution problems, which their disposal may otherwise cause [35]. These agricultural residues, such as wheat straw, represent an abundant, inexpensive, and readily available source of renewable lignocellulosic biomass. They are principally composed of cellulose, hemicelluloses, and lignin [36–38]. The former two components are hydrophilic, and the latter is hydrophobic. In other words, the cellulose and hemicelluloses, being more hygroscopic than lignin, are mainly responsible for moisture uptake [39]. These defects can be reduced considerably by chemical modification of its constituents. One of the methods is that the hydroxyl groups that are mainly responsible for its hygroscopicity, attached to cellulose, lignin, and hemicelluloses, can be changed to

hydrophobic groups by chemical modification, particularly for the production of novel materials for environmental friendly industrial utilization [35]. For example, Choi et al. demonstrated that cotton, milkweed, and kenaf had 1.5–3 times better sorption properties than polypropylene fibers [16]. Excellent oil sorption properties (Table 7.1.1) and high biodegradability of natural fibers make them particularly attractive as a possible alternative to the synthetic fibers.

Modification with acetic anhydride can substitute the hydroxyl groups of cell wall polymers from these vegetable products with acetyl groups, meanwhile improves the properties of these polymers so that they become hydrophobic. These modified absorbents have the characteristics of low cost, high capacity, and quick uptake and are easy to desorb by a simple squeezing method [36]. They could be used effectively to recover oil spilled in bodies of refining or heavy industrial wastewater and in the water of lakes, rivers, and oceans.

So far, chemical modification of lignocellulosics such as cereal straws using acetic anhydride is perhaps the simplest, safest, and cheapest method for preparing natural oil sorbents. Acetylation is a single-site reaction, that is, one acetyl per reacted hydroxyl group with one polymerization. In addition, acetyl groups are more hydrophobic than hydroxyl groups; therefore, replacing some of the hydroxyl groups with acetyl groups reduces the hydrophilic property of the cell wall polymers [32].

### 7.1.7 Types of Catalysts in Chemical Modification

Most of the procedures used to modify wood by acetylation developed over the years have complicated reaction schemes through the use of an organic cosolvent. Use of a solvent, which reduces the reaction rate because of dilution of modifiers, would require complicated separation procedures



**TABLE 7.1.1** Summary of Oil Sorbents and Their Sorption Capacities

Straw	Modifying reagent	Oil	Q <sub>max</sub> (g/g)	Ref.
Barley straw		Crude oil	15	[2]
Milkweed		Crude oil	40	[16]
Kapok		Engine oil	45	[26]
Sugarcane bagasse	Acetic anhydride	Machine oil	20.2	[35]
Wheat straw	Acetic anhydride	Machine oil	28.8	[40]
Cotton		Diesel	30.62	[41]
Kenaf		Diesel	7.16	[41]
Wheat straw	Hexadecyltrimethylammonium bromide	Emulsified oil		[42]
Cotton	Fatty acid	Vegetable/mineral oil	20	[18]

to recover the products after the reaction. Organic solvents are often harmful to humans and the environment. Therefore, it is the best to eliminate organic solvents in the reaction system [43]. On the other hand, the addition of catalysts has been proved to accelerate the reaction rate of acetic anhydride with wood because the reaction is acid or base catalyzed [44]. Pyridine-catalyzed acetylation is a standard method for the determination of hydroxyl compounds and other acylable substances. The mechanism involves a nucleophilic catalyst with the intermediate formation of the acylpyridium ion [45]. Although pyridine is an effective catalyst in such acylations, it is toxic, has an unpleasant odor, and is not suitable for use in large-scale reactions [46]. Hofle et al. [47] reported that a tertiary amine, 4-dimethylaminopyridine (DMAP), was much superior to pyridine as a catalyst for some synthetic acylations, such as typical acylation reactions for primary and secondary alcohols, and was an effective catalyst of analytical acylation by acetic anhydride, having a specific catalytic activity about  $10^4$  times greater than that of pyridine. Sun et al. reported that DMAP was the most effective catalyst for wheat straw acetylation compared with other three tertiary amine catalysts (Table 7.1.2). For many years, DMAP has been used as an acylation catalyst in chemical synthesis. Compared with pyridine, DMAP was found to be approximately  $10^4$  times more active when used as acylation catalyst [47], while it is too expensive and not commercially available reagent, which limits its industrial use.

In spite of a number of reports investigating various catalysts for acetylating in a solvent-free system (Table 7.1.3) [44, 48], the search for new catalysts is still actively pursued to address such problems as harsh reaction conditions; in addition, environmental and economic considerations have required the redesign of these commercially important processes. As a result, there is further scope to explore mild and efficient methods for

acetylation. Sugarcane bagasse (SCB) was esterified with acetic anhydride using N-bromosuccinimide (NBS) as a catalyst under mild conditions (Table 7.1.4) [35, 49]. The role of NBS is not clear, but a plausible explanation is that NBS might act as a source for  $\text{Br}^+$ , which in turn activates the carbonyl groups of acetic anhydride to produce the highly reactive acylating agent ( $\text{CH}_3\text{-CO-N-OCCH}_2\text{CH}_2\text{CO-}$ ). This acylating agent reacts with hydroxyl groups of SCB, which upon elimination of NBS produces acetylated SCB ( $\text{SCB-O-CO-CH}_3$ ) (Fig. 7.1.2) [35, 50]. Based on the study of acetylation of alcohols under mild reaction conditions, Karimi and Seradj [48] reported that NBS was an inexpensive and commercially available reagent and was a novel and highly effective catalyst for acetylation of alcohols under nearly neutral reaction conditions.

### 7.1.8 Agro-Based Sorbent Application

Coghan [51] reported that researchers at Virginia Polytechnic institute and State University in Blacksburg, Virginia, found that more oil can be absorbed with agro-based sorbents such as kenaf, cotton, and milkweed floss sorbent systems than that with commercial polypropylene fiber systems. The researchers reported that milkweed floss in particular was a good sorbent, absorbing approximately 40 times its weight in oil, compared with 10 times with polypropylene fibers. The great capability of milkweed floss to absorb oil was attributed to a waxy coating on the milkweed fiber floss, which retained 75% of its capability to absorb oil after three cycles of absorbing the fibers in oil and then mechanically removing the oil by squeezing. Agro-based absorbents are commercially used as oil absorbent socks and booms for oil spill cleanup.

The acetylated SCB can be used as a natural sorbent in oil spill cleanup, and its oil sorption capacity was much higher than that of the synthetic sorbents, indicating that a



**TABLE 7.1.2** The Effect of Different Catalysts on the Extent of Acetylation at 100 or 120 °C for Various Reaction Times [40]

Catalytic acetylation conditions			Acetylated wheat straw		
Temperature (°C)	Reaction time (min)	Catalyst (% dried straw)	Sample no.	WPG <sup>a</sup>	OA <sup>b</sup> (g oil/g straw)
120	40	DMAP <sup>c</sup> , 0	1	13.2	19.1
120	40	DMAP, 1	2	16.0	23.3
120	40	DMAP, 2	3	17.6	25.1
120	40	DMAP, 3	4	17.8	25.5
120	40	DMAP, 4	5	18.4	27.1
120	40	DMAP, 5	6	18.8	27.2
120	40	DMAP, 6	7	19.6	28.6
120	40	DMAP, 7	8	19.6	28.8
100	40	DMAP, 2	9	13.8	19.6
100	40	Pyridine, 2	10	8.4	12.4
100	40	MPI <sup>d</sup> , 2	11	10.2	15.4
100	40	MPO <sup>e</sup> , 2	12	10.0	14.8
100	40	DMAP, 6	13	15.4	23.1
100	40	Pyridine, 6	14	10.4	15.8
100	40	MPI, 6	15	10.6	15.9
100	40	MPO, 6	16	9.0	13.6
100	40	Without catalyst	17	7.8	12.0
120	60	DMAP, 2	18	17.8	25.6
120	90	DMAP, 2	19	18.0	26.0
120	120	DMAP, 2	20	18.2	26.4
120	180	DMAP, 2	21	18.2	26.6
120	360	DMAP, 2	22	18.4	27.5

<sup>a</sup>The weight percent gain by the product based on the starting dried wheat straw.<sup>b</sup>Oil absorptivity (g oil/g acetylated straw).<sup>c</sup>4-dimethylaminopyridine.<sup>d</sup>N-methylpyrrolidine.<sup>e,f</sup>N-methylpyrrolidinone.**TABLE 7.1.3** Summary of Main Catalysts Used in Cereal Straw Modification Reaction

Catalyst	Advantage	Disadvantage	Ref.
Pyridine	Nucleophilic mediated catalysis	Toxic, unpleasant odor, and not suitable for large scale reaction	[45]
4 dimethylaminopyridine	High efficiency	Too expensive, not commercially available	[47]
Cyltrimethylammonium bromide	Simplicity	Remove hemicellulose	[42]
N bromosuccinimide	High efficiency	Inexpensive and commercially available	[35]

**TABLE 7.1.4** Weight Percent Gain (WPG) of Acetylated Sugarcane Bagasse (SCB) Obtained with or without *N*-bromosuccinimide as a Catalyst Under Various Conditions [35]

Acetylation conditions				Acetylated bagasse		
Solid to liquid ratio <sup>a</sup> (g/ml)	Temperature (°C)	Reaction time (h)	Catalyst (% liquid, g/ml)	Sample no.	WPG <sup>b</sup>	OA <sup>c</sup> (g oil/g straw)
1:20	120	1.0	0	1	5.1	6.8
1:20	50	4.0	1	2	8.0	10.1
1:20	50	14.0	1	3	14.0	16.0
1:20	30	23.0	1	4	12.7	14.1
1:20	35	67.0	1	5	16.0	16.2
1:20	80	3.0	1	6	21.2	18.0
1:20	100	2.0	1	7	24.1	19.1
1:20	25	1.0	1	8	2.1	5.8
1:20	35	1.0	1	9	5.3	8.6
1:20	50	1.0	1	10	7.3	9.8
1:20	80	1.0	1	11	13.1	15.6
1:20	100	1.0	1	12	21.3	18.0
1:20	120	1.0	1	13	24.7	19.8
1:20	130	1.0	1	14	24.7	20.2
1:20	80	0.5	1	15	10.3	13.5
1:20	80	1.5	1	16	17.7	16.6
1:20	80	2.0	1	17	18.7	17.5
1:20	80	2.5	1	18	19.7	18.0
1:20	80	4.0	1	19	21.6	17.8
1:20	80	5.0	1	20	22.0	18.6
1:20	80	6.0	1	21	24.0	18.9

<sup>a</sup>Ratio of dried SCB (g)/acetic anhydride (ml).<sup>b</sup>WPG of SCB due to acetylation and it was calculated according to  $WPG(\%) = (\text{Weight gain}/\text{Original weight}) \times 100$ .<sup>c</sup>Oil absorptivity (g oil/g acetylated bagasse).

total or partial substitution of commercial synthetic oil sorbents by acetylated SCB could be beneficial in oil spill cleanup operations by improving the efficiency of oil sorption and by incorporation of other advantages such as biodegradability [35].

Using modified wheat straw to remove emulsified oil from water was reported by Fanta et al. [42]. The wheat straw was treated first by heating it in a sodium hydroxide (NaOH) solution and then subjecting it to an ion-exchange reaction with hexadecyltrimethylammonium bromide (CTAB). The researchers showed that sufficient NaOH was needed to disrupt the straw particles to produce a high surface area sorbent, but excess NaOH removed hemicelluloses. Minimizing hemicellulose removal is important

because the uronic acid substituents of hemicelluloses are responsible for much of the ion-exchange capacity of the straw. They thought that the simplicity of their straw-CTAB preparation made the process commercially attractive [25]. The acetylation significantly increased hydrophobic properties of the bagasse, and the oil sorption capacity of the acetylated bagasse obtained at 80 °C for 6 h was 1.9 times than the commercial synthetic sorbents. Adebajo and Frost studied the acetylation of cotton to develop hydrophobic, biodegradable, cellulosic materials for subsequent application in oil spill cleanup [52]. Acetylation also greatly improves biological resistance because of the reduced moisture sorption and substrate blocking of the reacted cell wall polymers. Hydroxyl groups are the most abundant

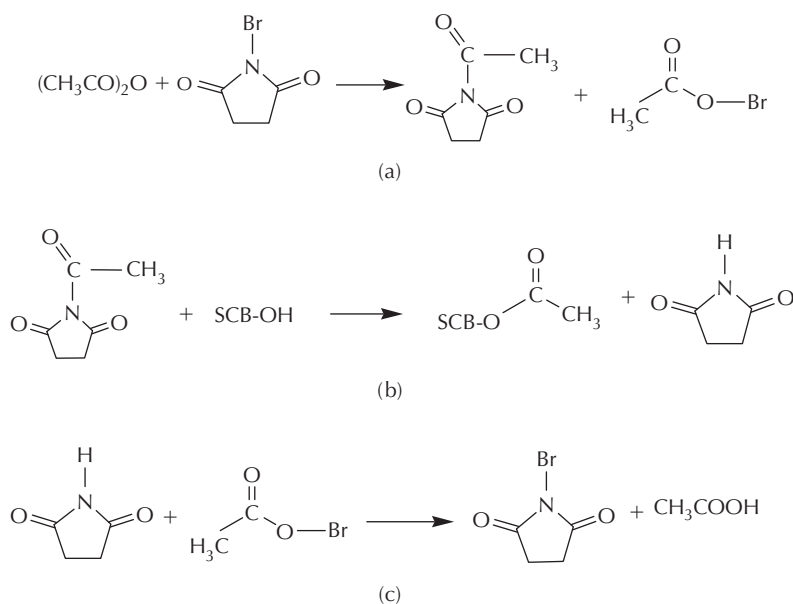


FIGURE 7.1.2 Mechanism of acetylation of SCB using NBS as a catalyst [35].

and reactive sites on the cell wall polymers of a lignocellulosic material such as SCB [53].

Wheat straw is one of the major agricultural by-products that are not used as industrial raw material on a significant scale except for only a minor portion that is reserved as animal feed, household fuel, or as raw materials for paper industry. Interestingly, straw is similar in chemical composition to wood. The predominant constituents of wheat straw are cellulose, hemicelluloses, and lignin [54]. The former two components are hydrophilic, and the latter is hydrophobic. However, straw is sparingly insoluble in water and organic solvents only partly because of the hydrogen bonds between polysaccharides and adhesion of lignin to the polysaccharides. However, it would be expected that wheat straw would react with acetic anhydride in a similar manner to wood and that the properties of acetylated wheat straw would be much the same as those of acetylated wood. Acetylated wheat straw could, therefore, represent an abundant, inexpensive, and renewable lignocellulosic biomass as a novel material for environmentally friendly industrial utilization. An example of this is potential usage as a natural sorbent in oil spill cleanup because acetylation increases the hydrophobic nature of the straw and gives a lower wettability but a higher oil absorptivity of the material [31].

Hydrophobic cotton fibers, obtained by acylation of cellulose with fatty acid using microwave radiations, have a high selective affinity for vegetable or mineral oil, fuel, and petroleum, in aqueous medium. Their sorption capacity (weight of liquid picked up by a given weight of sorbent) is about 20 g/g, after draining. They are reusable after simple squeezing, and their sorption capacity reaches a constant value, ca. 12 g/g. Moreover, this product is stable in water and is biodegradable [18].

## REFERENCES

- [1] T.R. Annunciado, T.H.D. Sydenstricker, S.C. Amico, Experimental investigation of various vegetable fibers as sorbent materials for oil spills, *Mar. Pollut. Bull.* 50 (2005) 1340–1346.
- [2] M. Hussein, A.A. Amer, A. El Maghraby, N.A. Taha, Availability of barley straw application on oil spill clean up, *Int. J. Environ. Sci. Technol.* 6 (2009) 123–130.
- [3] J.W. Smith, *The Control of Oil Pollution*, Graham and Trotman Publication, London, 1983.
- [4] J.R. Payne, C.R. Phillips, *Photochemistry of petroleum in water*, *Environ. Sci. Technol.* 19 (1985) 569–579.
- [5] R.E. Jordan, J.R. Payne, *Fate and Weathering of Petroleum Spills in the Marine Environment: A Literature Review and Synopsis*, Ann Arbor Science Publishers, Ann Arbor, MI, 1980.
- [6] P.F. Kingston, Long term environmental impact of oil spills, *Spill Sci. Technol. Bull.* 7 (2002) 53–61.
- [7] A.T. Nahla, *Study on the Use of Barley Straw for Oil Spill Cleanup*, A Thesis Presented to Graduate School, Faculty of Engineering, Alexandria University, Alexandria, 2008.
- [8] Q.F. Wei, R.R. Mather, A.F. Fotheringham, Oil removal from used sorbents using a biosurfactant, *Bioresour. Technol.* 96 (2005) 331–334.
- [9] H.M. Choi, Needle punched cotton nonwoven and other natural fibers as oil clean up sorbents, *J. Environ. Sci. Health A* 31 (1996) 1441–1457.
- [10] J. Pate, Cotton: a renewable resource for oil spill cleanups, *Nonwovens Ind.* 6 (1992) 32–36.
- [11] H.M. Choi, H. Kwon, J. Moreau, Cotton nonwovens as oil spill clean up sorbents, *Text. Res. J.* 63 (1993) 211–218.
- [12] J.G. Reynolds, P.R. Coronado, L.W. Hrubesh, Hydrophobic aerogels for oil spill cleanup intrinsic absorbing properties, *Energy Sources* 23 (2001) 831–843.
- [13] M. Toyoda, M. Inagaki, Sorption and recovery of heavy oils by using exfoliated graphite, *Spill Sci. Technol. Bull.* 8 (2003) 467–474.
- [14] Preus, Perlite Asphalt Fiber Compositions for Separating Hydrocarbon from Water, US Patent, 3855152, 1974.

- [15] Environment Protection Agency, Mechanical containment and recovery of oil following a spill, in: *Oil Spills*, EPA office of emergency and remedial response, Washington, DC, 2002, pp. 9–12.
- [16] H.M. Choi, R.M. Cloud, Natural sorbents in oil spill cleanup, *Environ. Sci. Technol.* 26 (1992) 772–776.
- [17] P. Schatzberg, U.S. Coast Guard Rep. No. 724110.1/2/1. U.S. Coast Guard Headquarters, Washington, DC, 1971.
- [18] G. Deschamps, H. Caruel, M.E. Borredon, C. Bonnin, C. Vignoles, Oil removal from water by selective sorption on hydrophobic cotton fibers. 1. Study of sorption properties and comparison with other cotton fiber based sorbents, *Environ. Sci. Technol.* 37 (2003) 1013–1015.
- [19] R. Johnson, T. Manjrekar, J. Halligan, Removal of oil from water surfaces by sorption on unstructured fibers, *Environ. Sci. Technol.* 7 (1973) 439–443.
- [20] M.M. Radetić, D.M. Jocić, P.M. Iovantić, Z.L. Petrović, H.F. Thomas, Recycled wool based nonwoven material as an oil sorbent, *Environ. Sci. Technol.* 37 (2003) 1008–1012.
- [21] M. Haussard, I. Gaballah, N. Kanari, P. de Donato, O. Barre's, F. Villieras, Separation of hydrocarbons and lipid from water using treated bark, *Water Res.* 37 (2003) 362–374.
- [22] I. Gaballah, D. Goy, E. Allain, G. Kilbertus, J. Thauront, Recovery of copper through decontamination of synthetic solutions using modified barks, *Metall. Mater. Trans. B* 28 (1997) 13–23.
- [23] W.S. Wan Ngah, M.A.K.M. Hanafiah, Removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents: a review, *Bioresour. Technol.* 99 (2008) 3935–3948.
- [24] A. Ghalambor, Evaluation and characterization of sorbents in removal of oil spills, Final report, Louisiana Oil Spill Coordinator's Office/Office of the Governor, Louisiana Applied Oil Spill Research and Development Program, Baton Rouge, LA, 1995, pp. 10–24.
- [25] R.M. Rowell, R.A. Young, J. Rowell, Filters, sorbents, and geotextiles, in: R.M. Rowell, R.A. Young, J.K. Rowell (Eds.), *Paper and Composites From Agro Based Resources*, Lewis Publishers, Boca Raton, FL, 1997, pp. 403–425.
- [26] T.T. Lim, X.F. Huang, Evaluation of kapok (*Ceiba pentandra* (L.) Gaertn.) as a natural hollow hydrophobic oleophilic fibrous sorbent for oil spill cleanup, *Chemosphere* 66 (2007) 955–963.
- [27] C.A.S. Hill, D. Jones, G. Strickland, N.S. Cetin, Kinetic and mechanistic aspects of the acetylation of wood with acetic anhydride, *Holzforschung* 52 (1998) 623–629.
- [28] R.M. Rowell, A.M. Tillman, L. Zhengtian, Dimensional stabilization of flakeboard by chemical modification, *Wood Sci. Technol.* 20 (1986) 83–95.
- [29] R.M. Rowell, Chemical modification of wood, *For. Prod. Abstr.* 6 (1983) 363–381.
- [30] R.M. Rowell, G.R. Esenther, D.D. Nicholas, T. Nilsson, Biological resistance of southern pine and aspen flakeboards made from acetylated flakes, *J. Wood Chem. Technol.* 7 (1987) 427–440.
- [31] F. Xu, R.C. Sun, The chemically modified cereal straw as efficient sorbents in oil spill cleanup, *J. Cell. Sci. Technol.* 11 (2003) 51–56.
- [32] R.M. Rowell, Opportunities for lignocellulosic materials and composites, *ACS Symp. Ser.* 476 (1992) 12–27.
- [33] G.S. Karr, X.Z. Sun, Strawboard from vapour phase acetylation of wheat straw, *Ind. Crops Prod.* 11 (2000) 31–41.
- [34] P.L. Brelid, R. Simonson, Acetylation of solid wood using microwave heating, Part II: experiments in laboratory scale, *Holz Roh Werkst.* 57 (1999) 383–389.
- [35] X.F. Sun, R.C. Sun, J.X. Sun, Acetylation of sugarcane bagasse using NBS as a catalyst under mild reaction conditions for the production of oil sorption active materials, *Bioresour. Technol.* 95 (2004) 343–350.
- [36] R.C. Sun, X.F. Sun, Structural and thermal characterization of acetylated rice, wheat, rye, and barley straws and poplar wood fibre, *Ind. Crops Prod.* 16 (2002) 225–235.
- [37] R.C. Sun, X.F. Sun, J.L. Ren, Fractional isolation and structural characterization of lignins isolated by alkali and alkaline peroxide from barley straw, *J. Agric. Food Chem.* 49 (2001) 5322–5330.
- [38] R.C. Sun, J. Tomkinson, P.L. Ma, S.F. Liang, Comparative study of hemicelluloses from rice straw by alkali and hydrogen peroxide treatments, *Carbohydr. Polym.* 42 (2000) 111–122.
- [39] R.M. Rowell, W.B. Banks, Tensile strength and toughness of acetylated pine and lime flakes, *Br. Polym. J.* 19 (1987) 482–497.
- [40] R.C. Sun, X.F. Sun, J.X. Sun, Q.K. Zhu, Effect of tertiary amine catalysts on the acetylation of wheat straw for the production of oil sorption active materials, *C.R. Chim.* 7 (2004) 125–134.
- [41] B.G. Lee, J.S. Han, R.M. Rowell, Oil sorption by lignocellulosic fibers, in: T. Sellers, N.A. Reichart (Eds.), *Kenaf Properties, processing and products*, Mississippi State University, Ag & Bio Engineering, Mississippi, 1999, pp. 423–433.
- [42] G.F. Fanta, T.P. Abbou, R.C. Burr, W.M. Doane, Ion exchange reactions of quaternary ammonium halides with wheat straw, preparation of oil absorbents, *Carbohydr. Polym.* 7 (1986) 97–109.
- [43] J.Z. Li, T. Furuno, S. Katoh, J. Uehara, Chemical modification of wood by anhydrides without solvents or catalysts, *Wood Sci.* 46 (2000) 215–221.
- [44] R.M. Rowell, A.M. Tillman, R. Simonson, A simplified procedure for the acetylation of hardwood and softwood Flakes for Flakeboard production, *J. Wood Chem. Technol.* 6 (1986) 427–448.
- [45] K.A. Connors, K.S. Albert, Determination of hydroxyl compounds by 4 dimethylaminopyridine catalyzed acetylation, *J. Pharm. Sci.* 62 (1973) 845–846.
- [46] C.A.S. Hill, N.S. Cetin, Z. Ozmen, Potential catalysts for the acetylation of wood, *Holzforschung* 54 (2000) 269–272.
- [47] G. Höfle, W. Steglich, H. Vorbrüggen, 4 Dialkylaminopyridines as highly active acylationcatalysts, *Angew. Chem. Int. Ed. Engl.* 17 (1978) 569–583.
- [48] C.A.S. Hill, H.P.S.A. Khalil, M.D. Hale, A study of the potential of acetylation to improve the properties of plant fibres, *Ind. Crops Prod.* 8 (1998) 53–63.
- [49] X.F. Sun, R.C. Sun, J.X. Sun, Oleoylation of sugarcane bagasse hemicelluloses using N bromosuccinimide as a catalyst, *J. Sci. Food Agric.* 84 (2004) 800–810.
- [50] B. Karimi, H. Seradj, N Bromosuccinimide (NBS), a novel and highly effective catalyst for acetylation of alcohols under mild reaction conditions, *Synlett* 4 (2001) 519–520.
- [51] A. Coghlan, Waxy weeds sup on oil slicks, *New Sci.* 134 (1992) 20–26.
- [52] M.O. Adebajo, R.L. Forest, Infrared and C MADS nuclear magnetic resonance spectroscopic study of acetylation of cotton, *Spectrochim. Acta A* 60 (2004) 449–453.
- [53] R.M. Rowell, R. Simonson, S. Hess, D.V. Plackett, D. Cronshaw, E. Dunningham, Acetyl distribution in acetylated whole wood and reactivity of isolated cell wall components to acetic anhydride, *Wood Fiber Sci.* 26 (1994) 11–18.
- [54] J.M. Lawther, R.C. Sun, W.B. Banks, Extraction, fractionation and characterization of structural polysaccharides from wheat straw, *J. Agric. Food Chem.* 43 (1995) 667–675.

# Modification of Cereal Straws as Natural Sorbents for Removing Metal Ions from Industrial Waste Water

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## 7.2.1 INTRODUCTION

Environmental pollution caused by toxic heavy metals is one of the most pressing problems in many densely populated cities worldwide. The industrial and domestic wastes responsible for various damages to the environment adversely affect the health of the human population. The major toxic metal ions hazardous to humans as well as other forms of life are chromium (Cr), iron (Fe), selenium (Se), vanadium (V), copper (Cu), cobalt (Co), nickel (Ni), cadmium (Cd), mercury (Hg), arsenic (As), lead (Pb), zinc (Zn), etc. Several events resulting from heavy metal contamination in the aquatic environment increased the awareness of the heavy metal toxicity. Among these, the Minamata tragedy in Japan because of methyl mercury contamination, and “Itai-Itai” disease because of toxicity of Cd poisoning in Jinsu river in Japan are well known [1, 2]. Therefore, it is necessary to remove these metals from industrial effluents before discharging aqueous waste into the environment. Under the current regulations, industries are obligated to treat waste water and to reduce the concentration of toxic metals to less than certain designated values before the discharge of industrial and urban household waste water [3].

Numerous methods exist for removing detrimental metal ions from aqueous solutions. The main techniques that have been utilized to reduce the concentration of the heavy metal ions in effluents include chemical precipitation, ion exchange, chemical oxidation, reverse osmosis, electro dialysis, and ultrafiltration [4–7]. Most of these methods have their own inherent limitations such as less efficiency, sensitive operating conditions, production of secondary sludge, and expensive disposal of the secondary sludge [8]. Another powerful technology is adsorption of heavy metals,

such as activated carbon (AC). AC has been recognized as a highly effective adsorbents for the removal of heavy metal ions from the concentrated and dilute metal-bearing effluents [9, 10]. However, the high cost of AC and its loss during the regeneration restrict its application [11]. For this reason, the use of low cost materials as sorbent for metal removal from wastewater has been highlighted.

Various agricultural residues such as cereal straws have been studied for their heavy metal uptake capacities and suitability to be used as a basis for adsorbent development [12–15]. The native exchange capacity and general sorptive characteristics of these materials derive from their constituent polymers: cellulose, hemicelluloses, lignin, pectin, and protein. Some of the advantages of using agricultural residues for wastewater treatment include simple technique, little processing requirement, good adsorption capacity, selective adsorption of heavy metal ions, low cost, free availability, and easy regeneration. However, the application of untreated plant wastes as adsorbents can also bring several problems such as low adsorption capacity, and high chemical oxygen demand (COD) and biological chemical demand (BOD), as well as total organic carbon (TOC) resulting from the release of soluble organic compounds contained in the agricultural residues [16, 17]. The increase of the COD, BOD, and TOC can cause depletion of dissolved oxygen content in water and can threaten the aquatic life. Therefore, agricultural residues need to be modified or treated before being applied for the decontamination of heavy metals.

The straw sources may be from wheat, rice, oat, barley, corn, and other grains or grass. Straw is cheap, easy to work with, and readily available in most localities [18]. In this chapter, an extensive list of adsorbents obtained from cereal straws was compiled and their methods of modification



were discussed. A comparison of adsorption efficiency between chemically modified and unmodified adsorbents was also reported.

## 7.2.2 MECHANISM OF METAL BIOSORPTION

The removal of metal ions from aqueous streams using agricultural residues is based on metal biosorption [19]. The process of biosorption involves a solid phase (sorbent) and a liquid phase (solvent) containing a dissolved species to be sorbed. Because of high affinity of the sorbent for the metal ion species, the latter is attracted and bound by rather complex process affected by several mechanisms involving chemisorption, complexation, adsorption-complexation on surface and pores, ion exchange, chelation, microprecipitation, adsorption by physical forces, entrapment in inter- and intrafibrillar capillaries, and spaces of the structural polysaccharides network as a result of the concentration gradient and diffusion through cell wall and membrane [20–22] (Fig. 7.2.1, [11]).

Agricultural residues are usually composed of cellulose, hemicelluloses, and lignin as the main constituents. Other components are extractives, lipids, proteins, simple sugars, starches, water, hydrocarbons, ash, and many more

compounds that contain a variety of functional groups present in the binding process. Cellulose is a crystalline homopolymer of glucose with  $\beta$ -(1 $\rightarrow$ 4) glycosidic linkage, and intra- and intermolecular hydrogen bonds [23]. Hemicelluloses are a heteropolymer of mainly xylose with  $\beta$ -(1 $\rightarrow$ 4) glycosidic linkage with other substances of acetyl feruloyl and glucuronyl groups [24]. Lignin is a three-dimensional polymer of aromatic compounds covalently linked with hemicelluloses in plant resources [25, 26]. The functional groups present in biomass molecules include acetamido, carbonyl, phenolic, amido, amino, sulfhydryl carboxyl, ester, and hydroxyl groups [27, 28]. These groups have the affinity for metal complexation. Some biosorbents are nonselective and bind to a wide range of heavy metals with no specific priority, whereas others are specific for certain types of metals depending on their chemical composition. The presence of various functional groups and their complexation with heavy metals during biosorption process has been reported by different research workers using spectroscopic techniques [8, 25, 29].

## 7.2.3 ADSORPTION MODELS

Isotherm adsorption models have been used in wastewater treatment to predict the ability of a certain adsorbent to remove a pollutant down to a specific discharge value.

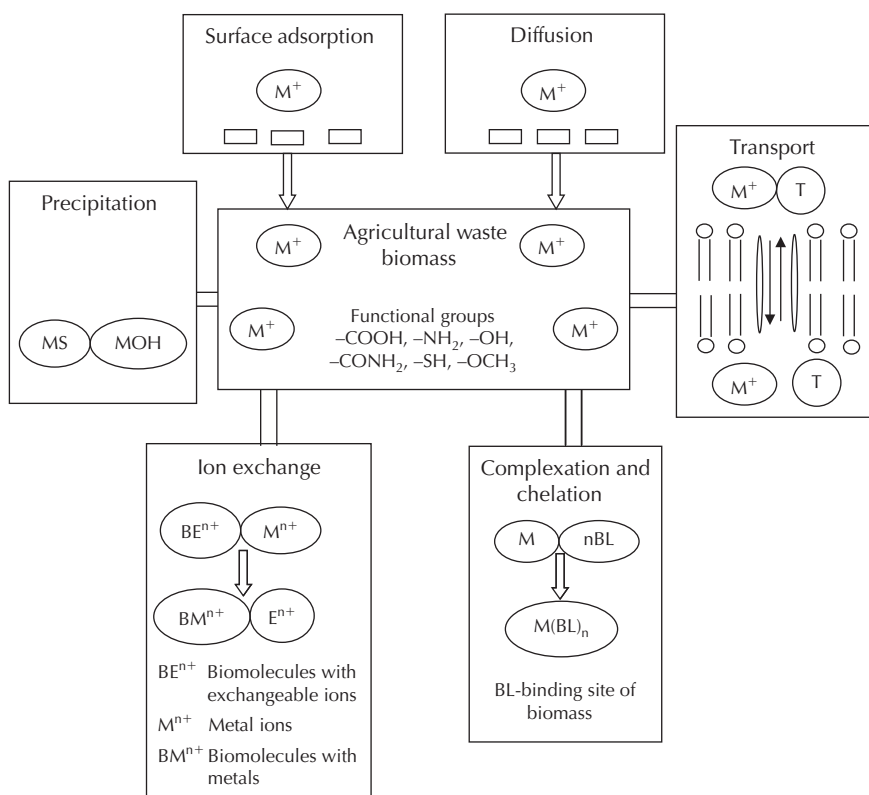


FIGURE 7.2.1 Plausible mechanism of biosorption [11].

When a mass of adsorbent and a wastewater stream are in contact for a sufficiently long time, equilibrium between the amount of pollutant adsorbed and the amount remaining in solution will develop [30]. For any system under equilibrium conditions, the amount of material adsorbed onto the media can be calculated using the mass balance of Eq. (7.2.1):

$$\frac{X}{M} = (C_o - C_e) \frac{V}{M}, \quad (7.2.1)$$

where  $X/M$  (typically expressed as milligram of pollutant per gram of media) is the mass of pollutant per mass of media,  $C_o$  is the initial pollutant concentration in solution,  $C_e$  is the concentration of the pollutant in solution after equilibrium has been reached,  $V$  is the volume of the solution to which the media mass is exposed, and  $M$  is the mass of the media.

Adsorption data for a wide range of adsorbate concentrations are most conveniently described by adsorption isotherms, such as the Langmuir or Freundlich isotherms [31, 32]. The general Langmuir model is defined by Eq. (7.2.2), in which  $K_L$  and  $\alpha_L$ , are the isotherm constants.

$$\frac{X}{M} = \frac{K_L C_e}{1 + \alpha_L C_e} \quad (7.2.2)$$

$K_L$  and  $\alpha_L$  values can be determined using linear regression. The Langmuir isotherm can be linearized to the following equation:

$$\frac{1}{X/M} = \frac{1}{K_L C_e} + \frac{\alpha_L}{K_L} \quad (7.2.3)$$

The general Freundlich equation is as follows:

$$\frac{X}{M} = K_F (C_e)^{1/n} \quad (7.2.4)$$

The Freundlich isotherm can also be linearized by the following:

$$\ln\left(\frac{X}{M}\right) = \ln K_F + \frac{1}{n} \ln C_e, \quad (7.2.5)$$

where  $K_F$  and  $n$  are adsorption capacity and affinity, respectively.

The Langmuir and Freundlich isotherm models are only applicable to batch adsorber systems, in which sufficient time is provided to allow equilibrium between the pollutant in solution and the pollutant adsorbed on the media to occur. During the flow through the adsorbent, many of the pollutants are expected to come into contact with active surface sites and, thus, be retained on the surface of the adsorbing media. Table 7.2.1 shows the Langmuir and Freundlich constants for the adsorption of Cu(II) and Cd(II) from aqueous solution onto rice husk (RH) and modified RH [33].

The bed depth-service time (BDST) model is based on the Bohart and Adams quasi-chemical rate law [35]. The assumption behind the equation (Bohart and Adams equation) is that the equilibrium is not instantaneous, and therefore, the rate of the sorption reaction is proportional to the fraction of sorption capacity still remaining on the media. The linearized BDST model equation is as follows [35–37]:

$$t_b = \frac{N_o}{1000 \varepsilon v C_o} D - \frac{1}{k C_o} \ln\left(\frac{C_o}{C_b}\right) \quad (7.2.6)$$

where  $t_b$  is the time until breakthrough (min),  $C_o$  is the initial concentration of pollutant ( $\text{mg}\cdot\text{L}^{-1}$ ),  $C_b$  is the breakthrough concentration of pollutant ( $\text{mg}\cdot\text{L}^{-1}$ ),  $v$  is the fluid velocity or loading rate ( $\text{m}\cdot\text{min}^{-1}$ ),  $\varepsilon$  is the porosity of the filter,  $k$  is quasi-chemical rate constant from Bohart and Adams theory ( $\text{L}\cdot\text{mg}\cdot\text{s}^{-1}$ ),  $N_o$  is the capacity of the media for each pollutant in a multicomponent solution (milligram of pollutant per cubic meter of filter volume), and  $D$  is the depth of the filter bed. Several empirical models proposed in the literature (Bohart Adams, Yan, Belter, and Chu models) were investigated to obtain the best fit of column data, describing in a simple manner the breakthrough curves [38].

## 7.2.4 METHODS OF CHEMICAL MODIFICATION

Unmodified cereal straws have a low heavy metal adsorption capacity as well as variable physical stability. Therefore, chemical modification of agricultural residues

**TABLE 7.2.1** Langmuir and Freundlich Constants for the Adsorption of Cu(II) and Cd(II) from Aqueous Solution onto Rice Husk (RH) and Modified RH (Adopted from [34])

Adsorbent	Heavy metal	$K_L$	$R_L^2$	$C_e$	$K_F$	$1/n$	$R_F^2$
Rice husk	Cu	2.48	0.996	0.143	0.693	0.288	0.968
	Cd	8.82	0.936	0.066	0.630	0.454	0.993
Modified rice husk	Cu	9.36	0.994	0.080	0.866	0.480	0.931
	Cd	11.03	0.993	0.069	1.265	0.495	0.952

$K_L$ ,  $C_e$  and  $R_L^2$  are the Langmuir constants.  $K_F$ ,  $n$ , and  $R_F^2$  are the Freundlich constants.

can be carried out to achieve adequate structural durability and efficient adsorption capacity for heavy metal ions [39]. Mechanisms and measurement of chemical modification were studied by Kunin et al. [34]. Chemical modification became popular and many researchers devised elaborate modification procedures [40]. Chemical modification can be used to vary certain properties of the component of agricultural residues, such as cellulose, its hydrophilic or hydrophobic characters, elasticity, water sorbency, adsorptive or ion-exchange capability, resistance to microbiological attack, and thermal resistance [41]. Hydroxyl groups are main functional groups in the agricultural residues. Functional groups may be attached to these hydroxyl groups by a variety of chemistries. The principle and main routes of direct hydroxyl groups modification in the preparation of adsorbent materials are esterification, etherification, halogenation, and oxidation (Table 7.2.2) and chemical grafting modification (Table 7.2.3) [42].

### 7.2.4.1 The Methods of Direct Chemical Modification

#### 7.2.4.1.1 Esterification

Carboxylic acids are known to improve water absorption in the fiber wall [57–61]. Modifying lignocellulosic fibers, cereal straws with cyclic anhydrides such as succinic, maleic, or phthalic anhydride have been pointed out as a way of introducing new material properties [62–66]. Gellerstedt and Gatenholm [67] introduced carboxylic acid functionalities to chemical pulp and chemical thermomechanical pulp by modification with succinic anhydride for various periods of time. Low et al. [44] used heat to convert citric acid (CA) to CA anhydride, which can further react with the cellulosic hydroxyl groups in wood pulp to form an ester linkage. This reaction introduced carboxyl functional groups to the cellulosic wood pulp material. The esterification process increases the carboxylic content of the fiber surface leading to a corresponding increase in the sorption of divalent metal ions. This modified pulp had Cu(II)- and Pb(II)-binding capacities of 24 and 83  $\text{mg}\cdot\text{g}^{-1}$ , respectively. Through a similar esterification reaction, Gaey and Marchetti [43], chemically modified wood pulp using succinic anhydride, in the presence of 1,2-dichloroethane, leading to the introduction of carboxyl groups. The Cd(II)-binding capacity of the modified sawdust was directly related to the acid value estimated by titration and could reach uptakes of up to 168  $\text{mg}\cdot\text{g}^{-1}$ .

#### 7.2.4.1.2 Etherification

Most cellulose ethers are prepared by reacting alkali cellulose with organic halides. Navarro et al. [45] modified the porous cellulose carriers through this type of etherification reaction. This was achieved by initially reacting the

cellulose carrier with sodium methylate to form alkali cellulose, which was subsequently reacted with the organic halide, epichlorohydrin, yielding reactive epoxy groups for further functionalization with polyethyleneimine as a chelating agent. The prepared adsorbent (Cell-PEI) had metal uptake affinities of 2.5, 38, and 12  $\text{mg}\cdot\text{g}^{-1}$  for Co(II), Cu(II), and Zn(II), respectively.

In another study, Saliba et al. [46] chemically modified the sawdust with amidoxime groups by reacting acrylonitrile (AN) with the sawdust through an etherification reaction to add cyano groups to the cellulose structure. These cyano groups were then amidoximated by reaction with hydroxylamine. This amidoximated sawdust had a high adsorption capacity of 246 and 188  $\text{mg}\cdot\text{g}^{-1}$  for Cu(II) and Ni(II), respectively.

#### 7.2.4.1.3 Halogenation

Halogenation represents another biomass-modification technique. Tashiro et al. [68] synthesized chlorodeoxycellulose by reacting cellulose powder with thionyl chloride in dimethylformamide solvent. The chlorodeoxycellulose was functionalized with ethylenediamine, thiourea, thiosemicarbazide, thioacetamide, hydroxylamine, and hydrazine. However, the synthesis was difficult because of the relatively low reactivity of cellulose with thionyl chloride. Aoki et al. [47] synthesized 6-deoxy-6-mercaptocellulose and its S-substituted derivatives from 6-bromo-6-deoxycellulose. Compared with chlorine, bromine has a higher reactivity with cellulose. Carboxyl, amino, isothiuronium, mercapto, and additional hydroxyl groups were introduced to cellulose and their adsorption behavior for metal ions was examined. The derivatives containing carboxyl groups because of the reaction with 2-mercaptobutanedioic acid had an adsorption capacity for Cu(II), Ni(II), and Pb(II) of 36, 9, and 104  $\text{mg}\cdot\text{g}^{-1}$ , respectively. The derivatives with amino and carboxyl groups because of the reaction with cysteine had an adsorption capacity for Cu(II), Ni(II), and Pb(II) of 22, 8, and 28  $\text{mg}\cdot\text{g}^{-1}$ , respectively.

#### 7.2.4.1.4 Oxidation

Reactive straw derivatives can also be prepared by oxidation and the subsequent functionalization of the oxidized lignocellulose. Maekawa et al. [48] prepared dialdehyde cellulose by periodate oxidation of cellulose. This dialdehyde cellulose was further oxidized using mildly acidified sodium chlorite. The 2,3-dicarboxy cellulose oxidized to nearly 100% oxidation level was completely soluble in water, but the 2,3-dicarboxy cellulose of 70% oxidation was largely insoluble in water. The latter was assessed for its heavy metal adsorption capability and uptake levels of 184 and 236  $\text{mg}\cdot\text{g}^{-1}$  were achieved for Ni(II) and Cu(II), respectively. Subsequently, Maekawa et al. [49] synthesized cellulose hydroxamic acid derivatives from dialdehyde cellulose obtained by the previous

periodate oxidation method, and their heavy metal adsorption capacities were investigated. These materials were capable of adsorbing  $246 \text{ mg}\cdot\text{g}^{-1}$  Cu(II) from aqueous solution. In another study, corn starch was oxidized (degree of substitution, DS 0.13–0.29) with sodium hypochlorite in the presence of 2,2,6,6-tetramethyl-1-piperdinyloxy and sodium bromide. The oxidized starch was effective for removing the  $\text{Cu}^{2+}$  [69].

Table 7.2.2 summarizes methods for direct modification of lignocellulose leading to heavy metal adsorbent materials. As an alternative, valuable properties deficient in native cellulose can be imparted to lignocellulose by grafting a second polymer as a long branch on the lignocellulose molecule. The following section will review the grafting possibilities.

#### 7.2.4.2 Chemical Grafting Modification

Graft copolymerization is a process in which the side chain grafts are covalently attached to a main chain of a polymer backbone to form a branched copolymer. The extent of polymerization graft is referred to as the degree of grafting (grafting yield) and is gravimetrically determined as the percentage of mass increase following copolymer preparation. Both the backbone and side chain grafts can be either homopolymer or copolymer. The active sites initiating polymerization reactions may be free radical or ionic chemical groups [70]. Various methodologies including high energy radiation, photochemical, and chemical initiation techniques have been used to activate or initiate the backbone cellulose polymer [71]. Initiation methods which generate free radicals have been received the greatest amount of attention because of their practicality. Free radicals are formed on the lignocellulosic molecules either by decomposition of a chemical initiator, ultraviolet (UV) light, or high energy radiation.

##### 7.2.4.2.1 Photografting

Photografting is a useful method for the introduction of various vinyl monomers onto cellulose materials [72]. The energy from the incident UV light is absorbed by the sensitizer, monomer, and/or polymer [73]. Kubota [74] introduced amidoxime groups to cellulose using a photografting technique. Initially, AN was grafted to the cellulose surface, subsequently the cyano groups were amidoximated by reaction with hydroxylamine. The ability of these cellulose amidoximated samples to adsorb Cu(II) were examined and the maximum adsorption capacity achieved was  $51 \text{ mg}\cdot\text{g}^{-1}$ . Kubota and Suzuki [72] also grafted AN to cellulose using the photografting technique. The resultant AN-grafted celluloses were subjected to reactions with triethylenetetraamine (Trien). The sample containing triethylenetetraamine groups showed an ability to adsorb Cu(II) to the extent of  $30 \text{ mg}\cdot\text{g}^{-1}$ .

##### 7.2.4.2.2 High Energy Radiation Grafting

High energy radiation ( $\gamma$ -rays or X-rays) can be used to graft various functional polymers to cellulose. Radioactive isotopes such as Cobalt-60 (Co-60) and Cesium-137 (Cs-137) are the main sources of  $\gamma$ -irradiation [70]. Bao et al. [75], recently, prepared an adsorbent resin by graft copolymerization of acrylic acid and acrylamide onto cellulose under microwave irradiation (electromagnetic radiation). This material was examined for its adsorption capacity for Cu(II) from wastewater. At optimal adsorption conditions, a maximum adsorption capacity for Cu(II) of  $49.6 \text{ mg}\cdot\text{g}^{-1}$  was achieved. Abdel-Aal et al. [76] used  $\gamma$ -irradiation for the graft copolymerization of acrylic acid onto wood pulp. The parameters affecting the abilities of the grafted wood pulp for removing Fe(III), Cr(III), Pb(II), and Cd(II) from aqueous solutions were investigated. The maximum uptake capacity was 7, 7, 4, and 6  $\text{mg}\cdot\text{g}^{-1}$  for Fe(III), Cr(III), Cd(II), and Pb(II), respectively.

##### 7.2.4.2.3 Chemical Initiation Grafting

The chemical initiation method has been extensively used and generally given higher graft yields [77]. Table 7.2.3 summarizes methods for chemical initiation grafting modification of lignocellulose leading to heavy metal adsorbent materials. Among the various redox-type initiators, ceric ion ( $\text{Ce(IV)}$ ) has gained great importance because of its high grafting efficiency [78]. With many of the aforementioned methods, the extent of ungrafted homopolymer is high in comparison with that from ceric (IV) ion-initiated grafting [79], in which homopolymerization of vinyl monomers are minimal. Previously, researchers have used this ceric-ion initiation method to graft functional polymers to cellulose in various forms. Hence, these reactive sites are introduced for either direct chelation of metal ions or subsequent reaction with a chelating agent for the same purpose.

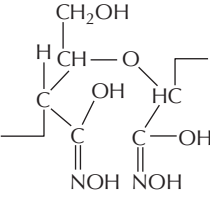
Raji and Anirudhan [50] grafted sawdust with acrylamide using the redox initiator potassium permanganate. The polyacrylamide-grafted sawdust was further functionalized with ethylenediamine to convert the material into an anion exchanger polymerized product. The maximum adsorption capacity of this material for Cr(VI) was  $45 \text{ mg}\cdot\text{g}^{-1}$ . The presence of diverse ions did not affect the adsorption performance. Shibi and Anirudhan [52] grafted acrylamide onto banana stalk using the ferrous ammonium nitrate/ $\text{H}_2\text{O}_2$  redox initiator system. The material was then aminated by reacting it with ethylenediamine and then refluxed with succinic anhydride to functionalize it with carboxylate groups. The maximum adsorption capacity of the adsorbent material for Hg(II) was  $138 \text{ mg}\cdot\text{g}^{-1}$  at  $30^\circ\text{C}$  which increased to  $210 \text{ mg}\cdot\text{g}^{-1}$  at  $60^\circ\text{C}$ . Bicak et al. [80] grafted polyacrylamide onto cotton cellulose using ceric ammonium sulfate as initiator and studied the removal of Hg(II) from aqueous solution and achieved a Hg(II) uptake level of  $12.5 \text{ mg}\cdot\text{g}^{-1}$ .

**TABLE 7.2.2** The Methods of Direct Chemical Modification and Associated Adsorption Capacities (Adopted from [42])

Modification reaction and materials	Modified chemicals (chelating group)	Structure	Adsorption capacity	pH Isotherm model	Ref.
Esterification					
Wood pulp	Succinic acid (Carboxy)	$\text{Cell}-\text{CH}_2-\text{O}-\text{C}(=\text{O})-\text{CH}_2-\text{CH}_2-\text{C}(=\text{O})-\text{OH}$	Cd(II) 169		[43]
Wood pulp	Citric acid (Carboxyl)	$\text{Cell}-\text{CH}_2\text{O}-\text{C}(=\text{O})-\text{CH}_2-\text{C}(\text{OH})(\text{COOH})-\text{CH}_2-\text{COOH}$	Cu(II) 24 Pb(II) 83	L	[44]
Etherification					
Cellulose carrier (aquacel)	(1) Sodium methylate (2) Epichlorohydrin (3) Polyethyleneimine (Amino)	$\text{Cell}-\text{CH}_2\text{O}-\text{CH}_2-\text{CHOH}-\text{CH}_2-(\text{NH}-\text{CH}_2-\text{CH}_2)_n$	Hg(II) 288	L	[45]
Wood sawdust	Acrylonitrile Hydroxylamine (Amino)	$\text{Cell}-\text{CH}_2\text{O}-\text{CH}_2-\text{C}(\text{NOH})=\text{NH}_2$	Cu(II) 246 Ni(II) 188	6.0 6.0	[46]
Halogenation cellulose powder	6 Bromo 6 deoxy cellulose + 2 Mercaptobutanedioic acid (Carboxyl)	$\text{Cell}-\text{CH}_2-\text{S}-\text{CH}(\text{COOH})-\text{CH}_2-\text{COOH}$	Cu(II) 36 Pb(II) 105 Ni(II) 0.93	5.0	[47]

(Continued)



Modification reaction and materials	Modified chemicals (chelating group)	Structure	Adsorption capacity	pH Isotherm model	Ref.
	Cysteine (Amino + Carboxyl)	$\text{Cell} - \text{CH}_2\text{S} - \text{CH}_2 - \underset{\text{COOH}}{\overset{\text{H}}{\text{C}}} - \text{NH}_2$	Cu(II) 22 Pb(II) 28 Ni(II) 8	5.0	[47]
	$\alpha$ Thioglycerol (Hydroxyl)	$\text{Cell} - \text{CH}_2\text{S} - \text{CH}_2 - \underset{\text{OH}}{\overset{\text{OH}}{\text{C}}} - \text{CH}_2$	Cu(II) 2 Pb(II) 6 Ni(II) 10	5.0	[47]
	3 Mercaptopropionic acid (Carboxyl)	$\text{Cell} - \text{CH}_2\text{S} - \text{CH}_2 - \underset{\text{COOH}}{\text{CH}_2}$	Cu(II) 24 Pb(II) 20 Ni(II) 1	5.0	[47]
	2 aminoethanethiol (Amino)	$\text{Cell} - \text{CH}_2\text{S} - \text{CH}_2 - \underset{\text{NH}_2}{\text{CH}_2}$	Cu(II) 6 Pb(II) 81 Ni(II) 5	5.0	[47]
Oxidation cellulose Powder	Sodium metaperiodate (Carboxyl)		Ni(II) 184 Cu(II) 236		[48]
Cellulose powder	Sodium metaperiodate Hydroxamic acid (Amino)		Cu(II) 246		[49]

**TABLE 7.2.3** The Methods of Chemical Initiation Grafting and Associated Adsorption Capacities (Adopted from [42])

Grafted adsorbent	Grafting agent	Structure	Adsorption capacity	pH isotherm model	Ref.
Sawdust	Acrylic acid (Carboxyl)	$\text{Cell} - (\text{CH}_2 - \underset{\text{COOH}}{\underset{ }{\text{CH}}})_{\text{mon}}$	Cu(II) 104	4.9	[43]
			Ni(II) 97	5.9	
			Cd(II) 168	5.7	
Sawdust	Acrylamide (Amino)	$\text{Cell} - (\text{CH}_2 - \underset{\text{CONH}_2}{\underset{ }{\text{CH}}})_{\text{mon}}$	Cr(VI) 45	3.0 F	[50]
Cotton cellulose	Acrylamide (Amino)	$\text{Cell} - (\text{CH}_2 - \underset{\text{CONH}_2}{\underset{ }{\text{CH}}})_{\text{mon}}$	Hg(II) 712	6.0	[51]
Banana stalk	(1) Acrylamide (2) Ethylenediamine (3) Succinic anhydride (Carboxyl)	$\text{Cell} - (\text{CH}_2 - \underset{\text{CONH} - (\text{CH}_2)_2 - \text{NHCO}(\text{CH}_2)_2 - \text{COOH}}{\underset{ }{\text{CH}}})_n$	Hg(II) 138	5.0 L	[52]
Sunflower stalks	(1) Acrylonitrile (2) Hydroxylamine (Amidoxime)	$\text{Cell} - \text{O} - \text{CH}_2 - (\text{CH}_2 - \underset{\text{NOH}}{\underset{  }{\overset{\text{NH}_2}{\text{C}}}})_{\text{mon}}$	Cu(II) 39	3.0 5.0	[53]
Cellulose	Glycidyl methacrylate (Imidazole)	$\begin{array}{c} \text{Cell} \\   \\ \text{C}=\text{O} \\   \\ \text{O} - \text{CH}_2 - \underset{\text{H}}{\underset{ }{\text{C}}} - \text{CH}_2 - \text{N} \end{array} \begin{array}{c} \text{OH} \\   \\ \text{CH} \end{array} \begin{array}{c} \diagup \diagdown \\ \text{C} \quad \text{C} \\ \diagdown \diagup \\ \text{N} \quad \text{N} \end{array}$	Cu(II) 68.5 Ni(II) 48.5 Pb(II) 75.8	4.0 5.5 L 4.0 6.0 4.0 6.0	[54 56]

In another study, Hashem [53] grafted sunflower stalks with AN using  $\text{KMnO}_4/\text{CA}$  redox initiator system. Amidoximation of the grafted stalks with hydroxylamine hydroxide in alkaline medium was carried out. The maximum uptake capacity of this adsorbent for  $\text{Cu(II)}$  was  $39 \text{ mg}\cdot\text{g}^{-1}$ . Gaey and Marchetti [43, 81] reported a method to graft polyacrylic chains onto sawdust using  $\text{KMnO}_4$  as initiator to attain an inexpensive adsorbent. The material had a high adsorption capacity of 104, 97, and  $168 \text{ mg}\cdot\text{g}^{-1}$  for  $\text{Cu(II)}$ ,  $\text{Ni(II)}$ , and  $\text{Cd(II)}$ , respectively. The monomer glycidyl methacrylate was also chosen by Bicak [82] as a reactive monomer for grafting because of the subsequent availability of its reactive epoxy groups for further functionalization. These epoxy groups are highly reactive to amines, alcohols, phenols, carboxylic acids, carboxylic anhydrides, Lewis acids and their complexes [51]. O'Connell et al. [54–56] grafted glycidyl methacrylate to a cellulose backbone and further functionalized the grafted product with the imidazole ligand. Adsorption levels on this material for  $\text{Cu(II)}$ ,  $\text{Ni(II)}$ , and  $\text{Pb(II)}$  reached 68.5, 48.5, and  $75.8 \text{ mg}\cdot\text{g}^{-1}$ , respectively. Navarro et al. [45] modified a porous cellulose material (aquacel) for heavy metal adsorption through graft polymerization of glycidyl methacrylate using ceric ammonium nitrate as the initiator, followed by the functionalization of the reactive epoxy groups present in poly (glycidyl methacrylate) with polyethyleneimine to introduce nitrogenous ligands. This material showed an adsorption capacity of 60, 20, and  $27 \text{ mg}\cdot\text{g}^{-1}$  for  $\text{Cu(II)}$ ,  $\text{Co(II)}$ , and  $\text{Zn(II)}$ , respectively, from aqueous wastewater.

## 7.2.5 CHEMICALLY MODIFIED STRAW

Modification of cereal straws can extract soluble organic compounds and enhance the chelating efficiency [17]. Modification methods using different kinds of modifying agents such as base solutions (sodium hydroxide, calcium hydroxide, sodium carbonate), mineral, and organic acid solutions (hydrochloric acid, nitric acid, sulfuric acid [ $\text{H}_2\text{SO}_4$ ], tartaric acid, CA, thioglycolic acid), organic compounds (ethylenediamine, formaldehyde, epichlorohydrin, methanol), oxidizing agent (hydrogen peroxide), dye (Reactive Orange 13), etc., for the purpose of removing soluble organic compounds, eliminating coloration of the aqueous solutions and increasing the efficiency of metal adsorption have been performed by many researchers. The modified straws and their maximum adsorption capacities are shown in Table 7.2.4.

### 7.2.5.1 Sugarcane Bagasse

Bagasse, an agricultural waste from sugar industry, has been found as low-cost metal adsorbent [83, 84]. Junior et al. [86] reported the use of succinic anhydride modified sugarcane bagasse for the treatment of  $\text{Cu}$ ,  $\text{Cd}$ , and  $\text{Pb}$

from aqueous solutions. Sugarcane bagasse consists of cellulose (43.6%), hemicelluloses (33.5%), and lignin (18.1%) [104]. The presence of these three biological polymers causes sugarcane bagasse rich in hydroxyl and phenolic groups, and these groups can be modified chemically to produce adsorbent materials with new properties. Ngah and Hanafiah [105] reported that the hydroxyl groups in sugarcane bagasse could be converted to carboxylic groups by using succinic anhydride. The carboxylic groups were later reacted with three different chemicals mainly  $\text{NaHCO}_3$ , ethylenediamine, and triethylenetetramine to produce adsorbent materials with new properties which showed different adsorption capacities for metal ions. It was found that sugarcane bagasse treated with ethylenediamine and triethylenetetramine showed a remarkable increase in nitrogen content compared to untreated sample, and triethylenetetramine-modified sugarcane bagasse has a higher increasing extent. The presence of amide group was also detected in ethylenediamine- and triethylenetetramine-modified sugarcane bagasses as a result of the reaction between  $\text{COOH}$  and  $\text{NH}_2$  groups. Kinetic studies showed that equilibrium time for adsorption of  $\text{Cu}$ ,  $\text{Cd}$ , and  $\text{Pb}$  onto tethylenediamine- and triethylenetetramine-modified sugarcane bagasses were slower than that for the adsorbent modified with  $\text{NaHCO}_3$ . Triethylenetetramine-modified sugarcane bagasse was the best adsorbent material for the removal of  $\text{Cd}$  and  $\text{Pb}$  as the adsorption capacities for both the metals were two times higher than the unmodified sugarcane bagasse. This was probably caused by the higher number of nucleophilic sites introduced in triethylenetetramine-modified sugarcane bagasse. However, when the sugarcane bagasse was modified with methanol, the modified adsorbent did not show a good uptake of  $\text{Cd}$ , and the maximum adsorption capacity was  $6.79 \text{ mg}\cdot\text{g}^{-1}$  [87]. In another study, through a fast, effective, and cheap methodology, it was possible to devise a strategy to introduce chelating functions (carboxylic acid and amine) to sugarcane bagasse. Modified sugarcane bagasse presented a maximum adsorption capacity of 139, 313, and  $313 \text{ mg}\cdot\text{g}^{-1}$  for  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ , and  $\text{Pb}^{2+}$ , respectively [89]. Orlando et al. [90] used microwave radiation to produce neutral bagasse chelating agents (BCA) by reaction of urea with the reactive sites present in bagasse such as hydroxyl and carboxylic groups. The prepared material had a  $\text{Cu(II)}$  and  $\text{Hg(II)}$  adsorption capacity of 76.2 and  $280.8 \text{ mg}\cdot\text{g}^{-1}$ , respectively.

Bagasse fly ash, a sugar industry waste, has been converted into an inexpensive and efficient adsorbent [90]. The product obtained was characterized and utilized for the removal of  $\text{Zn}$  from aqueous solutions over a wide range of initial metal-ion concentration ( $3.06 \times 10^{-4}$  to  $3.06 \times 10^{-3} \text{ M}$ ), contact time (24 h), adsorbent dose ( $5$ – $20 \text{ g}\cdot\text{L}^{-1}$ ), and pH (1.0–6.0). The removal of  $\text{Zn(II)}$  was 100% at low concentrations, whereas it was 60–65% at higher concentrations at an optimum pH of 4.0, using  $10 \text{ g}\cdot\text{L}^{-1}$  of

**TABLE 7.2.4** Summary of Modified Cereal Straw as Adsorbents for the Removal Metal Ions from Aqueous Solution

Adsorbent	Modifying agent(s)	Heavy metal	$Q_{\max}$ (mg·g <sup>-1</sup> )	Isotherm model	Ref.
Sugarcane bagasse	None	Cr(II)	13.4		[83]
Bagasse pith	None	Cd(II)	24.7	L	[84]
Sugarcane bagasse	Urea(Amino)	Cu(II)	76		[85]
		Hg(II)	280		
Sugarcane bagasse	Sodium bicarbonate	Cu(II)	114		[86]
		Pb(II)	196		
		Cd(II)	189		
	Ethylenediamine	Cu(II)	139		
		Pb(II)	164		
		Cd(II)	189		
	Triethylenetetramine	Cu(II)	133		
		Pb(II)	133		
		Cd(II)	313		
Sugarcane bagasse	Succinic anhydride	Cu(II)	114	L	[87]
		Pb(II)	189	L	
		Cd(II)	196	L	
	Succinic anhydride + amine	Cu(II)	139	L	
		Pb(II)	313	L	
		Cd(II)	313	L	
Sugarcane bagasse	Activated carbon	Zn(II)	31.1	L	[88]
		Cd(II)	38.0	L	
Bagasse fly ash	Unmodified	Zn(II)	13.2		[89]
Bagasse fly ash	Hydrogen peroxide	Pb(II)	2.5		[90]
		Cr(III)	4.4		
Rice husk	None	Cd(II)	2.0	L	[91]
Rice husk	None	Cd(II)	8.6	L	[92]
	NaOH	Cd(II)	20.2	L	
	Epichlorohydrin	Cd(II)	11.1	L	
	NaHCO <sub>3</sub>	Cd(II)	16.2	L	
	Ethylene diamine	Cr(VI)	23.4		
		Cu(II)			
Rice husk	Tartaric acid	Cu(II)	31.9		[93]
		Pb(II)	120.5		
Rice husk	NaOH	Cd(II)	7		[94]
	None	Cd(II)	4		
Rice whole with husk	None	Cr(III) + Cr(VI)	0.12		[95]

(Continued)

Adsorbent	Modifying agent(s)	Heavy metal	$Q_{\max}$ (mg·g <sup>-1</sup> )	Isotherm model	Ref.
Rice husk	None	Cr(III) + Cr(VI)	0.5		[95]
Rice husk ash	Heated at 300 °C	Cr(III) + Cr(VI)	19.1		[95]
	Heated at 600 °C	Cr(III) + Cr(VI)	6.5		
Rice hull	Ethlenediamine tetraacetic acid	Cu(II)	34.5		[96]
		Cr(III)	33.5		
		Ni(II)	32.5		
		Pb(II)	63.7		
Wheat Straw	Alkali treated	Cr(III)	1.9	L	[97]
	Insoluble straw xanthe	Cr(III)	3.9	L	
Wheat bran	NaCl	Cr(III)	93	L	
		Hg(II)	70	L	
		Pb(II)	62	L	
		Cd(II)	21	L	
		Cu(II)	15	L	
		Ni(II)	12	L	
Wheat bran	Sulfuric acid	Cu(II)	51.5	L	[98, 99]
	Sulfuric acid	Cd(II)	101	L	
Cormcorbs	None	Cd(II)	5.1	L	[100]
	Nitric acid (0.6 M)	Cd(II)	19.3	L	
	Citric acid (1 M)	Cd(II)	55.2	L	
Soybean hull	Untreatreated	Cu(II)	20.4		[101]
	NaOH	Cu(II)	24.4		
	NaOH + Citric acid (0.1 M)	Cu(II)	43.4		
	NaOH + Citric acid (0.3 M)	Cu(II)	76.3		
	NaOH + Citric acid (0.6 M)	Cu(II)	111.8		
	NaOH + Citric acid (0.9 M)	Cu(II)	135.3		
	NaOH + Citric acid (1.2 M)	Cu(II)	155.0		
Soybean straw	Water washed	Cu(II)	43.9	L	[102]
	Based washed	Cu(II)	42.9	L	
	Water washed + Citric acid (0.6 M)	Cu(II)	48.1	L	
	Based washed + Citric acid (0.6 M)	Cu(II)	48.7	L	
Barley straw	Untreated	Zn(II)	5.3		[103]
	Washed	Zn(II)	4.3		
	Washed + CaCO <sub>3</sub>	Zn(II)	4.1		



adsorbent in 6–8 h of equilibration time. The adsorption capacity of bagasse fly ash for Zn was found to be  $13.21 \text{ mg}\cdot\text{g}^{-1}$  at pH 4.0 and  $30^\circ\text{C}$ . Hydrogen peroxide is a good oxidizing agent and used to remove the adhering organic matter on the adsorbent. The performance of hydrogen peroxide treated bagasse fly ash for the removal of Pb and Cr was explored by Gupta and Jain [85]. It was found that hydrogen peroxide treated bagasse fly ash was able to remove Cr in a shorter period of time (60 min) when compared with Pb (80 min). The isotherm study also revealed that the maximum adsorption capacity for Cr was higher than Pb. However, the recorded values of maximum adsorption capacities for both the metals were low ( $2.50$  and  $4.35 \text{ mg}\cdot\text{g}^{-1}$  for Pb and Cr, respectively). The detailed mechanism of adsorption by the treated bagasse fly ash was not discussed, but it was thought that adsorption was controlled by film diffusion at lower metal concentration and particle diffusion at higher concentration of metal ions.

Mohan and Singh [107] studied potentiality of AC derived from bagasse for the removal of Cd(II) and Zn(II) from aqueous solutions in single as well as multimetal systems. Cd(II) adsorption was slightly more than Zn(II) and the increased sorption capacity was reported with increase in temperature. Adsorption on bagasse-based AC occurs through a film-diffusion mechanism at all concentrations. Using bagasse-based carbon, Ayyappan [88] studied adsorbent of Pb(II) under batch adsorption. Desorption of Pb(II) from sorbed carbon was achieved by eluting with  $0.1 \text{ M HNO}_3$ . Carbon was retrieved by washing with  $0.1 \text{ M CaCl}_2$  solution and reused. Similar studies were also carried out with Cr [88].

### 7.2.5.2 Rice Straw/Husks/Hulls

RH consists of 32.2%, cellulose; 21.3%, hemicelluloses; 21.4%, lignin; and 15.1%, mineral ash, as well as high percentage of silica in its mineral ash, which is approximately 96.3% [108, 109]. RH is insoluble in water, has good chemical stability, high mechanical strength and possesses a granular structure, making it a good adsorbent material for treating heavy metals from wastewater [91]. The removal of heavy metals by RH has been extensively reviewed by Chuah et al. [110]. The heavy metal ions studied include Cd, Pb, Zn, Cu, Co, Ni, and Au. RH can be used to treat heavy metals in the form of either untreated or modified using different modification methods. Roy et al. [111] demonstrated applicability of ground rice hulls for adsorption of heavy metals (As, Cd, Cr, Pb [ $>99\%$ ], and Sr [ $94\%$ ]). Maximum Cr(VI) removal ( $23.4 \text{ mg}\cdot\text{g}^{-1}$ ) by RH AC from aqueous solution was reported at pH 2.0 [112]. Daifullah et al. [113] used RH in removal of metals from a complex matrix containing six heavy metals (Fe, Mn, Zn, Cu, Cd, and Pb) and metal removal efficiency of sorbent was approximately 100%.

Hydrochloric acid [92], sodium hydroxide [92, 114], sodium carbonate, epichlorohydrin, and tartaric acid [93, 115] are commonly used in the chemical treatment of RH. Pretreatment of RHs can partly remove lignin, hemicelluloses, reduce cellulose crystallinity, and increase the porosity or surface area. In general, chemically modified or treated RH exhibits higher adsorption capacities on heavy metal ions than unmodified RH. For example, Kumar [92] reported that the treatment of RH with sodium hydroxide, sodium carbonate, and epichlorohydrin enhanced the adsorption capacity of Cd. The base treatment using NaOH, for instance, appeared to remove base-soluble materials on the RH surface that might interfere with its adsorption property. Tarley [94] found that adsorption of Cd increased by almost double when RH was treated with NaOH. The reported adsorption capacities of Cd(II) were 7 and  $4 \text{ mg}\cdot\text{g}^{-1}$  for NaOH-treated and unmodified RH, respectively.

Meanwhile, most of the acids used for treatment of plant wastes were in dilute form such as  $\text{H}_2\text{SO}_4$ , hydrochloric acid, and nitric acid. Dilute acid pretreatment using  $\text{H}_2\text{SO}_4$  can achieve high reaction rates and improve cellulose hydrolysis [116]. Concentrated acids are powerful agents for cellulose hydrolysis but they are toxic, corrosive, and must be recovered [117]. However, in some cases, hydrochloric acid-treated RH showed lower adsorption capacity of Cd than the untreated rice husk (URH) [92]. When RH is treated with hydrochloric acid, adsorption sites on the surface of RH will be protonated, leaving the heavy metal ions in the aqueous phase rather than being adsorbed on the adsorbent surface. Wong et al. [115] carried out an adsorption study of Cu and Pb on modified RH by various kinds of carboxylic acids (CA, salicylic acid, tartaric acid, oxalic acid, mandelic acid, malic, and nitrilotriacetic acid [NTA]) and it was reported that the highest adsorption capacity was achieved by modified RH with tartaric acid. Esterification with tartaric acid modified RH, but significantly reduced the uptake of Cu and Pb. The maximum adsorption capacities for Pb and Cu were reported as 108 and  $29 \text{ mg}\cdot\text{g}^{-1}$ , respectively. Effect of chelators on the uptake of Pb by tartaric acid modified RH was also studied. It was reported that higher molar ratios of chelators such as NTA and ethylenediamine tetraacetic acid (EDTA) caused significant suppressing effect on the uptake of Pb. Low et al. [96] also reported metal sorption enhancement by modifying rice hull with hydrochloric acid (ARH), sodium hydroxide (NaOH-RH), ethylenediamine tetraacetic acid (EDTA-RH), and nitrilotriacetic acid (NTA-RH). The order of metal sorption was  $\text{EDTA-RH} > \text{NTA-RH} > \text{NaOH-RH} > \text{RH} > \text{ARH}$ . EDTA was found to give the greatest enhancement. Sorption was pH dependent with greater uptake at higher pH value. Maximum sorption capacities of EDTA-modified rice hull were 8.86, 9.59, 8.76,  $28.65 \text{ mg}\cdot\text{g}^{-1}$  for Cu(II), Cr(III), Ni(II), and Pb(II), respectively. Dyestuff treated

rice hulls using Procion red and Procion yellow for the removal of Cr(VI), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), and Pb(II) were studied by Suemitsu et al. [95]. More than 80% of Cd(II), Pb(II), and Hg(II) ions were able to be removed by the two types of treated adsorbents, whereas Cr(VI) recorded the lowest percentage removal (<40%). Bakircioglu et al. [118] reported that the maximum adsorption capacities of URH, RH, RH ash heated at 300 °C (RHA-300) and 600 °C (RHA-600) were 0.12, 0.50, 19.09, and 6.49 mg·g<sup>-1</sup> for Cr(III) + Cr(VI), and 0.47, 294, 18.34, and 4.90 mg·g<sup>-1</sup> for Bi(V), respectively, showing RHA-300 as the most effective adsorbent.

### 7.2.5.3 Wheat Straw/Wheat Bran

Wheat straw is abundant and inexpensive in the world. The composition of wheat straw is cellulose 39.0% and hemicelluloses 38.7% [119], which is a natural biopolymer with ion-exchange property. This makes wheat straw a potentially good biosorbent for the treatment of wastewater containing heavy metal ions. Doan et al. [120] studied wheat straw particles as a biosorbent in a fixed-bed adsorber to remove Zn(II) and Ni(II). Acid pretreatment or prewetting of wheat straw can enhance the metal removal. The amount of metal ions adsorbed and the adsorption rate increased with the liquid pH from 4.0 to 7.0, with the liquid temperature from 30 to 35 °C. Kumar [121] used alkali-treated straw (ATS) and insoluble straw xanthate (ISX) to remove heavy metal. ISX consisting of 4.1% total sulfur was also applied for the removal of various metal ions, simultaneously. Removal of Cr(III) from aqueous solutions using ATS and ISX followed the Langmuir adsorption model, and both the materials showed significant Cr-removal efficiencies (>80%). In general, ISX is more effective toward the removal of many metal ions, simultaneously, without showing any selectivity. The differences in removal efficiencies between synthetic and actual waste could be primarily because of their different pH that can influence the ionic states of metal ions. In another study, carbonized wheat straw, prepared at different temperatures, were investigated for the adsorptive removal of Cr(VI) [122]. These carbonized materials at 800 °C at pH 2 from wheat straw were found to have a high affinity for Cr as represented by very high adsorption capacities of 1.67 and 1.68 mol·kg<sup>-1</sup>, respectively. This suggested that straw can be employed as an alternative to conventional adsorbents for the adsorption of Cr(VI). The equilibrium was reached within 1 h of contact time. The high adsorption capacity and very fast kinetic of both type of carbons studied make them promising alternatives for Cr(VI) removal.

Wheat bran, a byproduct of wheat milling industries, has been proved to be a good adsorbent for removal of many types of heavy metal ions such as Pb(II), Cu(II), and Cd(II). Farajzadeh [97] developed a new approach to obtain relatively high adsorption capacity utilizing wheat bran as

a natural metal adsorbent. Its efficiency was improved after the treatment with sodium chloride solution. Adsorption equilibrium was achieved in about 10 min for all studied cations. The adsorption capacities were 93, 70, 62, 21, 15, and 12 mg·g<sup>-1</sup> for Cr(III), Hg(II), Pb(II), Cd(II), Cu(II), and Ni(II), respectively. Treated wheat bran eliminated 89, 90, 96, 82, 97, 43, and 88% of Cr(III), Cd(II), Pb(II), Cu(II), Fe(III), Ni(II), and Hg(II) from solution, respectively. In addition, the application of a strong dehydrating agent like H<sub>2</sub>SO<sub>4</sub> had a significant effect on the surface area of the adsorbent, which eventually resulted in better efficiency of adsorption of Cu ions as reported by Özer et al. [98]. It was found that after treatment with H<sub>2</sub>SO<sub>4</sub>, wheat bran had a much higher surface area. The authors suggested that acid treatment caused changes in surface area by increasing the conversion of macropores to micropores. Maximum adsorption capacity for Cu(II) ions was reported as 51.5 mg·g<sup>-1</sup> (at pH 5) and the equilibrium time of adsorption was achieved in 30 min. Özer et al. [99] conducted a study on the removal of Pb ions by H<sub>2</sub>SO<sub>4</sub> treated wheat bran. It was reported that maximum Pb removal (82.8%) occurred at pH 6 after 2 h of contact time. Three isotherm models including Langmuir, Freundlich, and Redlich Peterson were analyzed for determining the maximum adsorption capacity of wheat bran. Based on the nonlinear plots, it was found that adsorption fitted well to the Redlich Peterson than Langmuir and Freundlich models. The Langmuir plots indicated that maximum adsorption capacities increased with an increase in temperature (79.37 mg·g<sup>-1</sup> at 60 °C and 55.56 mg·g<sup>-1</sup> at 25 °C). The decrease in the values of  $\Delta G^\circ$  suggested that adsorption was more favorable at higher temperatures and adsorption was endothermic in nature. The kinetic study showed that Pb adsorption could be described well with *n*th-order kinetic model. Özer [123] also examined the H<sub>2</sub>SO<sub>4</sub>-treated wheat bran for Cd-ion removal from the aqueous solution. After 4 h of contact time, the maximum adsorption capacity that could be achieved for Cd was 101 mg·g<sup>-1</sup> at pH 5. Therefore, the order of maximum removal of the above three metals follows: Cd(II) > Pb(II) > Cu(II).

### 7.2.5.4 Corncobs

Corncobs are widely available and inexpensive macromolecular waste. Seea et al. [124] studied the thermochemical reaction between corncob and CA and obtained a modified corncob, which had a large cation exchange capacity than natural corncob. Vaughan et al. [100] chemically analyzed the corncobs and found that it mostly consisted of 38.4%, cellulose; 40.7%, hemicelluloses; and 9.1%, lignin. They modified ground corncobs with either 0.6 M CA or phosphoric acid to improve their natural adsorption capacity. The effect of a combination of washing and modification treatment was tested for corncob adsorption with five different metal ions (Cd, Cu, Pb, Ni, and Zn)

individually or in a mixed solution containing each metal at a 20 mM concentration. Results were also compared with those of commercial resins Amberlite IRC-718, Amberlite 200, Duolite GT-73, and carboxymethylcellulose (CMC). Modified corncobs showed the same adsorption efficiency as Duolite GT-73 for Cd, Cu, Ni, and Zn ions and had greater adsorption than CMC for Ni and Zn ions. For mixed metals, the modified corncobs exhibited the same adsorption efficiency as Duolite GT-73 for Cd and Cu ions, and the same or higher adsorption than Amberlite IRC-718, Amberlite 200, and Duolite GT-73. The adsorption capacity of modified corncobs for Cu and Pb ions was equivalent to that of Duolite GT-73 but was lower than that of Amberlite IRC-718 or Amberlite 200. Lehrfeld [125] reported the carboxylate (maleate, succinate, and phthalate), phosphate, sulfate groups, and glyoxylic acid were incorporated onto the polysaccharide matrix of corncobs. The modified corncobs exhibited calcium-binding capacities in the following order: sulfate > succinate > phthalate > phosphate > maleate > glyoxylic acid > native corncob. Besides carbonization at high temperature, an adsorbent can be activated by chemical treatment using a concentrated acid. This method was demonstrated by Khan and Wahab [126] in the study of adsorption of Cu by concentrated  $\text{H}_2\text{SO}_4$ -treated corncobs. It was reported that on the treatment of corncobs with  $\text{H}_2\text{SO}_4$  and heated at 150 °C, the  $\text{pH}_{\text{zpc}}$  of the adsorbent reduced from 5.2 (untreated) to 2.7 (treated), and the functional groups present in the adsorbent are mainly oxygen-containing groups such as OH, COOH and  $\text{COO}^-$ . The maximum adsorption capacity obtained from Langmuir isotherm was 31.45  $\text{mg}\cdot\text{g}^{-1}$ . Adsorption was more favored at higher pH value (4.5) because of low-competing effect of protons for the adsorption sites. The effect of interfering ions such as Zn(II), Pb(II), and Ca(II) was also studied. It was noticed that Cu-removal efficiency was reduced by 53, 27, and 19% in the presence of Pb(II), Ca(II), and Zn(II), respectively. Regeneration study indicated that  $\text{H}_2\text{SO}_4$ -treated corncobs could be regenerated by acidified hydrogen peroxide solution and as much as 90% Cu could be recovered.

Oxidation of corncob by CA and nitric acid was carried out by Leyva-Ramos et al. [127]. Upon oxidation of corncob, a significant increase in the surface area of the adsorbent was observed. An increase in the amount of oxygen found in corncob was because of more oxygenated groups being introduced on the adsorbent surface after oxidation. After oxidation, a higher proportion of acidic sites (carboxylic, phenolic, and lactonic) were detected, which resulted in a reduction in the  $\text{pH}_{\text{ZPC}}$  value. It was also reported that the adsorption capacities for CA and nitric acid-oxidized corncob were much higher than unmodified corncob. In addition, Billon et al. [128] showed the feasibility of esterified derivatives of corncobs used to clean back condensates from high-pressure industrial boilers. Copper ions were retained by these modified natural polymers within working pH and

temperature conditions even at very low concentrations as  $10^{-12} \text{ mol}\cdot\text{L}^{-1}$ . Acrylamide polymers or derivatives were not successful for such applications. The succinic anhydride derivatives of corncobs were demonstrated to be more efficient than the maleic anhydride derivatives.

### 7.2.5.5 Soybean Straw/Hull

Soybean hulls are a byproduct of soybean processing and occur in great abundance on an annual basis. Marshall et al. [129] studied the removal of Zn(II) ions by treating soybean hulls with 0.1 M NaOH and 0.1 M HCl solutions. The results obtained from acid and base treatments were compared with water washed adsorbents. For soybean hulls, a 26% increase in adsorption capacity was observed after NaOH treatment compared with water washing. However, the capacity was greatly reduced (about 78%) after washing the adsorbent with HCl. The authors reported that both adsorbents contain pectin substances consisting primarily of galacturonic acid and *O*-methyl esters. Upon treatment with NaOH, the *O*-methyl esters are converted to methanol and additional galacturonic acid. The adsorbent surfaces will have more negative charge sites or greater number of metal-adsorption sites, hence greater metal adsorption capacity. Acid-washed hulls, however, cause the adsorbent surface to be protonated, causing Zn(II) ions more difficult to be adsorbed [92].

CA is a low-cost material used extensively in the food industry. Corn byproducts were derivatized by Wing [130] with CA and observed considerable improvement in Cu(II) binding. When heated, CA dehydrates to yield a reactive anhydride which can react with the sugar hydroxyl groups to form an ester linkage. The introduced free carboxyl groups of CA increase the net negative charge on the straw fiber, thereby increasing its binding potential for cationic contaminants [101, 130–132]. Marshall [101] treated the soybean hulls with 0.1 N NaOH and modified with different concentrations (0.1–1.2 M) of CA at 120 °C for 90 min. CA-modified hulls had adsorption capacities for Cu(II) from 0.68 to 2.44  $\text{mmol}\cdot\text{g}^{-1}$ , which was much higher than that of unmodified hulls (0.39  $\text{mmol}\cdot\text{g}^{-1}$ ). The total negative charge for these hulls also increased with increasing CA concentration and was about twice the Cu-ion adsorption capacity at all CA concentrations. For NaOH-treated and CA-modified hulls, increasing the temperature from 25 to 60 °C appeared to have no effect on the rate of Cu-ion removal from the solution. CA modification of soybean hulls greatly enhanced the metal-ion removal efficiency and resulted in a product with possible commercial potential for metal-ion remediation. Previously, Wartelle et al. [131] reported an interesting finding in which a linear relationship between total negative charge and amount of Cu ions adsorbed was observed for 12 types of agricultural byproducts (sugarcane bagasse, peanut shells, macadamia nut hulls, rice hulls, cottonseed hulls, corn cob, soybean



hulls, almond shells, almond hulls, pecan shells, English walnut shells, and black walnut shells) after modification with CA. It was found that after washing with base (NaOH) and modified with CA, the total negative charge of all 12 types of agricultural byproducts increased significantly. Among the 12 adsorbents, soybean hulls (a low-density material) showed the highest Cu uptake and had a high total negative charge value, which could be explained by the increase in carboxyl groups after thermochemical reaction with CA. However, nutshells (high-density materials) such as English walnut shells and black walnut shells displayed low total negative charge values indicating low number of carboxyl groups. As a result of the high bulk density, the lignin in nutshells may block or allow little penetration of CA to reactive sites and, hence, lower Cu-ion uptake was observed. However, the process must be optimized for it to be cost effective. In this regard, Marshall [133] developed a wash procedure to remove the nonreacted or residual CA after soybean hull modification, to maximize the amount of nonreacted acid removed, but minimize the subsequent effect on the product's ability to adsorb Cu(II), and to determine whether recycling the nonreacted acid in the modification process, along with the unused acid, can produce a product with similar Cu-ion adsorption compared with the use of unused acid only. The study indicated that at least for a limited number of exposures (three) to both unused and reused acid, this assumption is valid. This information also has future scale-up considerations. They determined that about 55% of the original CA was nonreactive and could be removed by water washing. Because this acid can be reused, significant cost savings can be achieved by recycling acid rather than using unused acid for each batch of soybean hulls.

Soybean straw is a lignocellulosic agricultural stalk. It's plentiful, inexpensive, and renewable. Over 16 million metric tons of soybeans are produced annually in China [134], but most of soybean straw is arbitrarily discarded or set on fire. It consists of 38%, cellulose; 16%, hemicelluloses; 16%, lignin; 0.83%, nitrogen; and 6%, ash [102]. Zhu et al. [102] studied the soybean straw which was water or base (0.1 M NaOH) washed and CA modified to enhance its natural adsorption capacity. The amount of Cu(II) adsorbed by soybean straw increased after modification with CA, regardless of whether the samples were base washed or water washed. This was because of the increase in carboxyl groups imparted onto the straw by reaction with CA. The adsorption capacities increased when the solution pH increased from 2 to 6 and reached the maximum value at pH 6. The Cu(II) uptake increased and adsorption percentage of the Cu(II) decreased with the increase in initial Cu(II) concentration from 1 mM to 20 mM. Both the Langmuir and Freundlich adsorption isotherms were tested, and the Freundlich model fitted much better than the Langmuir model. It was found that CA-based soybean straw has the highest adsorption capacity of the four kinds of pretreated soybean straw.

### 7.2.5.6 Other Straw/Hulls

Larsen [103] studied the efficiency of barley straw in removing heavy metals from solution in capacity and in column experiments. In the capacity experiments the efficiency of the straw was compared to that of AC, pine sawdust, and  $\text{CaCO}_3$ . It was found that 1 g of straw was able to adsorb amounts of Zn, Cu, Pb, Ni, and Cd ranging from 4.3 to 15.2 mg. One gram of activated carbon removed from 6.2 to 19.5 mg and pine sawdust from 1.3 to 5.0 mg, and  $\text{CaCO}_3$  from 1.6 to 19.8 mg. In column experiment, a column packed of barley straw and saturated with Cu was regenerated with 1 liter of acid ( $\leq 1.0$  N HCl) and could be reused at least of five times. From 1.6 to 19.8 mg, the different metals were precipitated by  $\text{CaCO}_3$ . It may be concluded that this material can remove considerable amounts of different heavy metals from solutions, and the straw can be regenerated and reused several times. AC produced from oat hulls in adsorbing As(V) was tested in a batch reactor [135]. The results indicated that the adsorptive capacity of AC was affected by an initial pH value, with adsorption capacity decreasing from 3.09 to 1.57 mg of As per gram of AC when the initial pH values increased from 5 to 8. A modified linear driving force model conjugated with a Langmuir isotherm was created to describe the study of kinetics. The results showed that the rapid adsorption and slow adsorption exist simultaneously when AC is used to remove As(V).

## 7.2.6 SUMMARY

In recent years, increasing costs and environmental considerations associated with the use of commercial adsorbents, has led to a significant body of research work aimed at developing new low-cost adsorbents derived from renewable resources. In this chapter, the advantages of using the cereal straws as the basis for new adsorbent design lie primarily in its high abundance, low cost, and relatively easily chemical modification. Carboxylic and hydroxyl functional groups on surface of cereal straws have high affinity for heavy metal ions. The most common chemicals used for treatment of cereal straws are acids and bases. Chemical modification, in general, improves the adsorption capacity of adsorbents, probably because of the higher number of active binding sites after modification, better ion-exchange properties, and formation of new functional groups that favors metal uptake. Many of the adsorption interactions between the modified cereal straws and heavy metals have been characterized by the Langmuir approach or in a lesser number of cases by the Freundlich model of adsorption. The process of biosorption requires further investigation in the direction of modeling, regeneration of biosorbent and recovery of metal ions and immobilization of the cereal straws for enhanced efficiency and recovery. Most of the reported studies are performed in the batch

process, which also gives a platform for the designing of the continuous flow systems with industrial applications at the commercial level. Furthermore, research focusing on metal recovery and regeneration of cereal straws should be carried out to make the process economically viable at industrial scale in the future.

## REFERENCES

- [1] T. Kjellstrom, K. Shiroishi, P.E. Erwin, Urinary beta./sub 2/ microglobulin excretion among people exposed to cadmium in the general environment, *Environ. Res.* 13 (1977) 318 344.
- [2] L. Friberg, C.G. Elinder, *Encyclopedia of Occupational Health*, third ed., International Labor Organization, Geneva, 1985.
- [3] S.K. Shany, J. Reedijk, Coordination chemistry of chelating resins and ion exchangers, *Coord. Chem. Rev.* 59 (1984) 1 139.
- [4] B.M. Braukmann, Industrial solution amenable to biosorption, in: B. Volusky (Ed.), *Biosorption*, CRC Press, Boca Raton, FL, 1990.
- [5] L. Zhang, L. Zhao, Y. Yu, C. Chen, Removal of lead from aqueous solution by non living *Rhizopus nigricans*, *Water Res.* 32 (1998) 1437 1444.
- [6] J.W. Patterson, *Industrial Wastewater Treatment Technology*, second ed., Butterworth, Stoneham, MA, 1985.
- [7] J.L. Gardea Torresdey, K.J. Tiemann, V. Armendariz, L. Bess Oberto, R.R. Chianelli, J. Rios, J.G. Parsons, G. Gamez, Characterization of chromium (VI) binding and reduction to chromium (III) by the agricultural byproduct of *Avena monida* (oat) biomass, *J. Hazard. Mater.* 80 (2000) 175 188.
- [8] S.S. Ahluwalia, D. Goyal, Removal of heavy metals from waste tea leaves from aqueous solution, *Eng. Life Sci.* 5 (2005) 158 162.
- [9] M. Hosea, B. Greene, R. McPherson, M. Henzl, M.D. Alexander, D.W. Darnall, Accumulation of elemental gold on alga *Chlorella vulgaris*, *Inorg. Chim. Acta* 123 (1986) 161 165.
- [10] T. Horikoshi, A. Nakajima, T. Sakaguchi, Studies on the accumulation of heavy metal elements in biological systems, XIX: accumulation of uranium by microorganisms, *Appl. Microbiol. Biotechnol.* 12 (1981) 90 96.
- [11] D. Sud, G. Mahajan, M.P. Kaur, Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions A review, *Bioresour. Technol.* 99 (2008) 6017 6027.
- [12] S. Al Asheh, Z. Duvnjak, Adsorption of copper by canola meal, *J. Hazard. Mater.* 4 (1996) 83 93.
- [13] S.H. Lee, J.W. Yang, Removal of copper in aqueous solution by apple wastes, *Sep. Sci. Technol.* 32 (1997) 1371 1387.
- [14] V.K. Gupta, D. Mohan, S. Sharma, Removal of lead from wastewater using bagasse fly ashes a sugar industry waste material, *Sep. Sci. Technol.* 33 (1998) 1331 1343.
- [15] B. Yu, Y. Zhang, A. Shukla, S.S. Shukla, K.L. Dorris, The removal of heavy metals from aqueous solutions by sawdust adsorptions removal of lead and comparison of its adsorption with copper, *J. Hazard. Mater.* B84 (2001) 83 94.
- [16] A. Nakajima, T. Sakaguchi, Recovery and removal of uranium by using plant wastes, *Biomass* 21 (1990) 55 63.
- [17] I. Gaballah, D. Goy, E. Allain, G. Kilbertus, J. Thauront, Recovery of copper through decontamination of synthetic solutions using modified barks, *Metall. Mater. Trans. B* 28 (1997) 13 23.
- [18] B. Raton, *Paper and Composites from Agro Based Resources*, Lewis Publishers, CRC Press, 1997, pp. 403 425.
- [19] B. Volesky, Z.R. Holan, Biosorption of heavy metals, *Biotechnol. Progr.* 11 (1995) 235 250.
- [20] M.C. Basso, E.G. Cerrella, A.L. Cukierman, Lignocellulosic materials as potential biosorbents of trace toxic metals from wastewater, *Chem. Res.* 41 (2002) 3580 3585.
- [21] K.V. Sarkanen, C.H. Ludwig, *Lignins Occurrence, Formation, Structure and Reactions*, Wiley Interscience, New York, 1971.
- [22] S. Qaiser, A.R. Saleemi, M.M. Ahmad, Heavy metal uptake by agro based waste materials, *Environ. Biotechnol.* 10 (2007) 409 416.
- [23] E. Demirbas, M. Kobya, E. Senturk, T. Ozkan, Adsorption kinetics for the removal of chromium (VI) from aqueous solutions on the activated carbons prepared from agricultural wastes, *Water SA* 30 (2004) 533 539.
- [24] U.K. Garg, M.P. Kaur, V.K. Garg, Removal of Nickel (II) from aqueous solution by adsorption on agricultural waste biomass using a response surface methodological approach, *Bioresour. Technol.* 99 (2008) 1325 1331.
- [25] U.K. Garg, M.P. Kaur, V.K. Garg, D. Sud, Removal of hexavalent Cr from aqueous solutions by agricultural waste biomass, *J. Hazard. Mater.* 140 (2007) 60 68.
- [26] K. Freudenberg, Lignin: its constitution and formation from p hydroxycinnamyl alcohols, *Science* 148 (1965) 595 600.
- [27] T.J. Beveridge, R.G.E. Murray, Sites of metal deposition in the cell wall of *Bacillus subtilis*, *J. Biotechnol.* 141 (1980) 876 887.
- [28] V.K. Gupta, I. Ali, Utilization of bagasse fly ash (a sugar industry waste) for the removal of copper and zinc from wastewater, *Sep. Purif. Technol.* 18 (2000) 131 140.
- [29] C.R.T. Tarley, M.A.Z. Arruda, Biosorption of heavy metals using rice milling byproducts, characterization and application for removal of metals from aqueous effluents, *Chemosphere* 54 (2004) 987 995.
- [30] A. Demirbas, Heavy metal adsorption onto agro based waste materials: a review, *J. Hazard. Mater.* 157 (2008) 220 229.
- [31] I. Langmuir, The constitution and fundamental properties of solids and liquids, *Am. Chem. Soc.* 38 (1916) 2221 2295.
- [32] H. Freundlich, Over the adsorption in solution, *J. Phys. Chem.* 57 (1906) 385 470.
- [33] D.O. Cooney, *Adsorption Design for Wastewater Treatment*, Lewis Publishers, Boca Raton, FL, 1999.
- [34] R. Kunin, A. Tavares, G. Wilber, New developments in the use of ion exchangers and adsorbents as precoat filter, in: D. Naden, M. Streat (Eds.), *Ion Exchange Technology*, Horwood, Chichester, UK, 1984.
- [35] J. Goel, K. Kadirvelu, C. Rajagopal, V.K. Garg, Removal of lead (II) by adsorption using treated granular activated carbon: batch and column studies, *J. Hazard. Mater.* 125 (2005) 211 220.
- [36] G. McKay, *Use of Adsorbents for the Removal of Pollutants from Wastewaters*, CRC Press, Boca Raton, FL, 1996.
- [37] P. Lodeiro, R. Herrero, M.E. Sastre de Vicente, The use of protonated *Sargassum muticum* as biosorbent for cadmium removal in a fixed bed column, *J. Hazard. Mater.* 137 (2006) 244 253.
- [38] S.A. Ong, C.E. Seng, P.L. Lim, Kinetics of adsorption of Cu (II) and Cd (II) from aqueous solution on rice husk and modified rice husk, *Electron. J. Environ. Agric. Food Chem.* 6 (2007) 1764 1774.
- [39] S. Kamel, E.M. Hassan, M. El Sakhawy, Preparation and application of acrylonitrile grafted cyanoethyl cellulose for the removal of copper(II) ions, *J. Appl. Polym. Sci.* 100 (2006) 329 334.
- [40] M. Morita, M. Higuchi, I. Sakata, Binding of heavy metal ions by chemically modified Woods, *J. Appl. Polym. Sci.* 34 (1987) 1013 1023.



- [41] D.J. McDowall, B.S. Gupta, V.T. Stannett, Grafting of vinyl monomers to cellulose by ceric ion initiation, *Prog. Polym. Sci.* 10 (1984) 1–50.
- [42] D.W. O'Connell, C. Birkinshaw, T.F. O'Dwyer, Heavy metal adsorbents prepared from the modification of cellulose: a review, *Bioresour. Technol.* 99 (2008) 6709–6724.
- [43] M. Gaey, V. Marchetti, A. Clement, B. Loubinoux, P. Gerardin, Decontamination of synthetic solutions containing heavy metals using chemically modified sawdusts bearing polyacrylic acid chains, *J. Wood Sci.* 46 (2000) 331–333.
- [44] K.S. Low, C.K. Lee, S.M. Mak, Sorption of copper and lead by citric acid modified wood, *Wood Sci. Technol.* 38 (2004) 629–640.
- [45] R.R. Navarro, K. Sumi, M. Matsumura, Improved metal affinity of chelating adsorbents through graft polymerization, *Water Res.* 33 (1999) 2037–2044.
- [46] R. Saliba, H. Gauthier, R. Gauthier, Adsorption of heavy metal ions on virgin and chemically modified lignocellulosic materials, *Adsorpt. Sci. Technol.* 23 (2005) 313–322.
- [47] N. Aoki, K. Fukushima, H. Kurakata, M. Sakamoto, K. Furuhashi, 6 Deoxy 6 mercaptocellulose and its S substituted derivatives as sorbents for metal ions, *React. Funct. Polym.* 42 (1999) 223–233.
- [48] E. Maekawa, T. Koshijima, Properties of 2, 3 dicarboxy cellulose combined with various metallic ions, *J. Appl. Polym. Sci.* 29 (1984) 2289–2297.
- [49] E. Maekawa, T. Koshijima, Preparation and characterisation of hydroxamic acid derivatives and its metal complexes derived from cellulose, *J. Appl. Polym. Sci.* 40 (1990) 1601–1613.
- [50] C. Raji, T.S. Anirudhan, Batch Cr(IV) removal by polyacrylamide grafted sawdust: kinetics and thermodynamics, *Water Res.* 32 (1998) 3772–3780.
- [51] T. Kondo, A. Ishizu, J. Nakano, Preparation of glycidyl celluloses from completely allylated methylcellulose and tri *O* allylcellulose, *J. Appl. Polym. Sci.* 37 (1989) 3003–3009.
- [52] I.G. Shibi, T.S. Anirudhan, Synthesis, characterisation, and application as a mercury (II) sorbent of banana stalk polyacrylamide grafted copolymer bearing carboxyl groups, *Ind. Eng. Chem. Res.* 41 (2002) 5341–5352.
- [53] A. Hashem, Amidoximated sunflower stalks (ASFS) as a new adsorbent for removal of Cu(II) from aqueous solution, *Polym. Plast. Technol.* 45 (2006) 35–42.
- [54] D.W. O'Connell, C. Birkinshaw, T.F. O'Dwyer, A chelating cellulose adsorbent for the removal of Cu(II) from aqueous solutions, *J. Appl. Polym. Sci.* 99 (2006) 2888–2897.
- [55] D.W. O'Connell, C. Birkinshaw, T.F. O'Dwyer, A modified cellulose adsorbent for the removal of Ni(II) from aqueous solutions, *J. Chem. Technol. Biotechnol.* 81 (2006) 1820–1828.
- [56] D.W. O'Connell, C. Birkinshaw, T.F. O'Dwyer, Removal of lead(II) from aqueous solutions using a modified cellulose adsorbent, *Adsorpt. Sci. Technol.* 24 (2006) 337–347.
- [57] A.M. Scallan, The effect of acidic groups on the swelling of pulps: a review, *Tappi J.* 66 (1983) 73–75.
- [58] A.M. Scallan, The Accomodation of Water Within Pulp Fibres, BPBIF 6th Fundamental Res. Symposium, Oxford, England, 1977, pp. 9–29.
- [59] T. Lindström, Chemical factors affecting the behaviour of fibres during papermaking, *Nordic. Pulp Pap. Res. J.* 4 (1992) 181–192.
- [60] R.A. Young, Cross linked cellulose and cellulose derivatives, in: P.K. Chatterjee (Ed.), *Absorbency*, Elsevier, Amsterdam, 1985, pp. 217–255.
- [61] G. Carlsson, Surface Composition of Wood Pulp Fibres: Relevance to Wettability, Sorption and Adhesion, Dissertation Thesis, Royal Institute of Technology, Stockholm, Sweden, 1996, pp. 8–10.
- [62] Y. Zhang, B. Sjögren, P. Engstrand, M. Htun, Determination of charged groups in mechanical pulp fibres and their influence on pulp properties, *J. Wood Chem. Technol.* 14 (1994) 83–102.
- [63] P. Luner, Y.J. Zhou, P. Caluwe, B. Tekin, Wet reinforcing of paper and board by novel crosslinking chemicals, in: C.F. Baker (Ed.), *Products of Papermaking*, Surrey, Pira International, UK, 1993, pp. 1045–1072.
- [64] J.C. Roberts, J. Tatham, Succinylation of Pulps, the Chemistry of Papermaking Conference, Pira International, Manchester, UK, 1992.
- [65] H. Matsuda, Preparing and utilisation of esterified woods bearing carboxylic groups, *Wood Sci. Technol.* 21 (1987) 75–88.
- [66] K. Garves, Rapid esterification of cellulose with dicarboxylic acid anhydrides, *Tappi J.* 55 (1972) 263–263.
- [67] F. Gellerstedt, P. Gatenholm, Surface properties of lignocellulosic fibers bearing carboxylic groups, *Cellulose* 6 (1999) 103–121.
- [68] T. Tashiro, Y. Shimura, Removal of mercuric ions by systems based on cellulose derivatives, *J. Appl. Polym. Sci.* 27 (1982) 747–756.
- [69] D.K. Kweon, J.K. Choi, E.K. Kim, S.T. Lim, Adsorption of divalent metal ions by succinylated and oxidized corn starches, *Carbohydr. Polym.* 46 (2001) 171–177.
- [70] M.M. Nasef, A.H. El Sayed, Preparation and applications of ion exchange membranes by radiation induced graft copolymerisation of polar monomers onto non polar films, *Prog. Polym. Sci.* 29 (2004) 499–561.
- [71] A. Bhattacharya, B.N. Misra, Grafting: a versatile means to modify polymers: techniques, factors and applications, *Prog. Polym. Sci.* 29 (2004) 767–814.
- [72] H. Kubota, S. Suzuki, Comparative examinations of reactivity of grafted celluloses prepared by ultra violet and ceric salt initiated graftings, *Eur. Polym. J.* 31 (1995) 701–704.
- [73] J.A. Harris, J.C. Arthur, J.H. Carra, Photoinitiated polymerization of glycidyl methacrylate with cotton cellulose, *J. Appl. Polym. Sci.* 22 (1978) 905–915.
- [74] H. Kubota, Y. Shigehisa, Introduction of amidoxime groups into cellulose and its ability to adsorb metal ions, *J. Appl. Polym. Sci.* 56 (1995) 147–151.
- [75] X.Z. Bao, W. Peng, Z. Tong, Y.C. Chun, S. Jing, Preparation and adsorption performance of a callulosic adsorbent resin for copper (II), *J. Appl. Polym. Sci.* 99 (2006) 2951–2956.
- [76] S.E. Abdel Aal, Y. Gad, A.M. Dessouki, The use of wood pulp and radiation modified starch in wastewater treatment, *J. Appl. Polym. Sci.* 99 (2006) 2460–2469.
- [77] S.R. Shukla, A.R. Athalye, Graft copolymerisation of glycidyl methacrylate onto cotton cellulose, *J. Appl. Polym. Sci.* 54 (1994) 279–288.
- [78] D.N.S. Hon, Graft Copolymerization of Lignocellulosic Fibers, American Chemical Society Symposium Series, 187, Washington, DC, USA, 1982.
- [79] K.C. Gupta, K. Khandehar, Graft copolymerisation of acrylamide methylacrylate co monomers onto cellulose using ceric ammonium nitrate, *J. Appl. Polym. Sci.* 86 (2002) 2631–2642.
- [80] N. Bicak, S.F. Senkel, Sorbitol modified poly(N glycidyl styrene sulfonamide) for removal of boron, *J. Appl. Polym. Sci.* 68 (1998) 2113–2119.
- [81] V. Marchetti, P. Gerardin, P. Tekely, B. Loubinoux, Graft copolymerization of acrylic acid onto sawdusts using KMnO<sub>4</sub> as initiator, *Holzforschung* 52 (1998) 654–660.
- [82] N. Bicak, D.C. Sherrington, B.F. Senkal, Graft copolymer of acrylamide onto cellulose as mercury selective sorbent, *React. Funct. Polym.* 41 (1999) 69–76.
- [83] D.C. Sharma, C.F. Foster, A preliminary examination into the adsorption of hexavalent chromium using low cost adsorbents, *Bioresour. Technol.* 47 (1994) 257–264.

- [84] K.A. Krishnan, T.S. Anirudhan, Removal of Cd (II) from aqueous solutions by steam activated sulphurised carbon prepared from sugarcane Bagasse pith: kinetics and equilibrium studies, *Water SA* 29 (2003) 147–156.
- [85] V.K. Gupta, C.K. Jain, I. Ali, M. Sharma, V.K. Saini, Removal of cadmium and nickel from wastewater using bagasse fly ash – a sugar industry waste, *Water Res.* 37 (2003) 4038–4044.
- [86] O.K. Junior, L.V.A. Gurgel, J.C.P. de Melo, V.R. Botaro, T.M.S. Melo, R.P. de Freitas Gil, L.F. Gil, Adsorption of heavy metal ion from aqueous single metal solution by chemically modified sugarcane bagasse, *Bioresour. Technol.* 98 (2006) 1291–1297.
- [87] S.C. Ibrahim, M.A.K.M. Hanafiah, M.Z.A. Yahya, Removal of cadmium from aqueous solution by adsorption on sugarcane bagasse, *Am. Eurasian J. Agric. Environ. Sci.* 1 (2006) 179–184.
- [88] R. Ayyappan, A. Carmalin Sophia, K. Swaminathan, S. Sandhya, Removal of Pb (II) from aqueous solution using carbon derived from agricultural wastes, *Process Biochem.* 40 (2005) 1293–1299.
- [89] O. Karnitz Jr, L.V.A. Gurgel, J.C. sar Perin de Melo, V.R. Botaro, T.M. rcia Sacramento Melo, R.P. de Freitas Gil, L.F. de ric Gil, Adsorption of heavy metal ion from aqueous single metal solution by chemically modified sugarcane bagasse, *Bioresour. Technol.* 98 (2007) 1291–1297.
- [90] V.K. Gupta, S. Sharma, Removal of zinc from aqueous solutions using bagasse fly ash a low cost adsorbent, *Ind. Eng. Chem. Res.* 42 (2003) 6619–6624.
- [91] M. Ajmal, R.A.K. Rao, S. Anwar, J. Ahmad, R. Ahmad, Adsorption studies on rice husk: removal and recovery of Cd(II) from wastewater, *Bioresour. Technol.* 86 (2003) 147–149.
- [92] U. Kumar, M. Bandyopadhyay, Sorption of cadmium from aqueous solution using pretreated rice husk, *Bioresour. Technol.* 97 (2006) 104–109.
- [93] K.K. Wong, C.K. Lee, K.S. Low, M.J. Haron, Removal of Cu and Pb from electroplating wastewater using tartaric acid modified rice husk, *Process Biochem.* 39 (2003) 437–445.
- [94] C.R.T. Tarley, S.L.C. Ferreira, M.A.Z. Arruda, Use of modified rice husks as a natural solid adsorbent of trace metals: characterization and development of an on line preconcentration system for cadmium and lead determination by FAAS, *Microchem. J.* 77 (2004) 163–175.
- [95] R. Suemitsu, R. Uenishi, I. Akashi, M. Nakano, The use of dyestuff treated rice hulls for removal of heavy metal ions from waste water, *J. Appl. Polym. Sci.* 31 (1986) 75–83.
- [96] K.S. Low, C.K. Lee, S.Y. Wong, P.L. Tang, Metal sorption enhancement of rice hull through chemical modification, *Environ. Technol.* 21 (2000) 1239–1244.
- [97] M.A. Farajzadeh, A.B. Monji, Adsorption characteristics of wheat bran towards heavy metal cations, *Sep. Purif. Technol.* 38 (2004) 197–207.
- [98] A. Özer, D. Özer, The adsorption of copper(II) ions onto dehydrated wheat bran (DWB): determination of equilibrium and thermodynamic parameters, *Process Biochem.* 39 (2004) 2183–2191.
- [99] A. Özer, H.B. Pirincci, The adsorption of Cd(II) ions on sulfuric acid treated wheat bran, *J. Hazard. Mater. B* 137 (2006) 849–855.
- [100] T. Vaughan, C.W. Seo, W.E. Marshall, Removal of selected metal ions from aqueous solution using modified corncobs, *Bioresour. Technol.* 78 (2001) 133–139.
- [101] W.E. Marshall, L.H. Wartelle, D.E. Boler, M.M. Johns, C.A. Toles, Enhanced metal adsorption by soybean hulls modified with citric acid, *Bioresour. Technol.* 69 (1999) 263–268.
- [102] B. Zhu, X.F. Tong, D. Zhang, Adsorption of copper ions from aqueous solution by citric acid modified soybean straw, *J. Hazard. Mater.* 153 (2008) 300–308.
- [103] V.J. Larsen, H.H. Schierup, The use of straw for removal of heavy metals from wastewater, *J. Environ. Qual.* 10 (1981) 188–193.
- [104] J.X. Sun, R.C. Sun, X.F. Sun, Y.Q. Su, Fractional and physico chemical characterization of hemicelluloses from ultrasonic irradiated sugarcane bagasse, *Carbohydr. Res.* 339 (2004) 291–300.
- [105] W.S.W. Ngah, M.A.K.M. Hanafiah, Removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents: a review, *Bioresour. Technol.* 99 (2008) 3935–3948.
- [106] U.S. Orlando, A. Baes, W. Nishijima, M. Okada, Preparation of chelating agents from sugarcane bagasse by microwave radiation as an alternative ecologically benign procedure, *Green Chem.* 4 (2002) 555–557.
- [107] D. Mohan, K.P. Singh, Single and multi component adsorption of Cd(II) and Zn(II) using activated carbon derived from bagasse – an agricultural waste, *Water Res.* 36 (2002) 2304–2318.
- [108] I.A. Rahman, J. Ismail, Preparation and characterization of a spherical gel from a low cost material, *J. Mater. Chem.* 3 (1993) 931–934.
- [109] I.A. Rahman, J. Ismail, H. Osman, Effect of nitric acid digestion on organic materials and silica in rice husk, *J. Mater. Chem.* 7 (1997) 1505–1509.
- [110] T.G. Chuah, A. Jumariah, S. Katayon, S.Y. Thomas Choong, Rice husk as a potentially low cost biosorbent for heavy metal and dye removal: an overview, *Desalination* 175 (2005) 305–316.
- [111] D. Roy, P.N. Greenlaw, B.S. Shane, Adsorption of heavy metals by green algae and ground rice hulls, *J. Environ. Sci. Health A* 28 (1993) 37–50.
- [112] N.R. Bishnoi, M. Bajaj, N. Sharma, A. Gupta, Adsorption of Cr (VI) on activated rice husk carbon and activated alumina, *Bioresour. Technol.* 91 (2004) 305–307.
- [113] A.A.M. Daifullah, B.S. Girgis, H.M.H. Gad, Utilization of agrowaste residues (rice husk) in small waste water treatment plants, *Mater. Lett.* 57 (2003) 1723–1731.
- [114] Y. Guo, S. Yang, W. Fu, J. Qi, R. Li, Z. Wang, H. Xu, Adsorption of malachite green on micro and mesoporous rice husk based active carbon, *Dyes Pigm.* 56 (2003) 219–229.
- [115] K.K. Wong, C.K. Lee, K.S. Low, M.J. Haron, Removal of Cu and Pb by tartaric acid modified rice husk from aqueous solutions, *Chemosphere* 50 (2003) 23–28.
- [116] A. Esteghlalian, A.G. Hashimoto, J.J. Fenske, M.H. Penner, Modeling and optimization of the dilute sulfuric acid pretreatment of corn stover, poplar and switchgrass, *Bioresour. Technol.* 59 (1997) 129–136.
- [117] M.V. Sivers, G. Zacchi, A techno economical comparison of three processes for the production of ethanol from pine, *Bioresour. Technol.* 51 (1995) 43–52.
- [118] Y. Bakircioglu, D. Bakircioglu, S. Akman, Solid phase extraction of bismuth and chromium by rice husk, *J. Trace Microprobe Tech.* 21 (2003) 467–478.
- [119] F. Xu, C.F. Liu, Z.C. Geng, J.X. Sun, R.C. Sun, B.H. He, L. Lin, S.B. Wu, J. Je, Characterization of graded organosolv hemicelluloses from wheat straw, *Polym. Degrad. Stab.* 91 (2006) 1880–1886.
- [120] H.D. Doan, A. Lohi, V.B.H. Dang, T. Dang Vu, Removal of Zn<sup>+2</sup> and Ni<sup>+2</sup> by adsorption in a fixed bed of wheat straw, *Process Saf. Environ.* 86 (2008) 259–267.

- [121] A. Kumar, N.N. Rao, S.N. Kaul, Alkali treated straw and insoluble straw xanthate as low cost adsorbents for heavy metal removal preparation, characterization and application, *Bioresour. Technol.* 71 (2000) 133–142.
- [122] R. Chand, T. Watari, K. Inoue, T. Torikai, M. Yada, Evaluation of wheat straw and barley straw carbon for Cr(VI) adsorption, *Sep. Purif. Technol.* 65 (2009) 331–336.
- [123] A. Özer, Removal of Pb(II) ions from aqueous solutions by sulfuric acid treated wheat bran, *J. Hazard. Mater. B* 141 (2006) 753–761.
- [124] D.J. Sessa, R.E. Wing, in: D.J. Sessa, J. Willett (Eds.), *Paradigm for Successful Utilization of Renewable Resources*, AOCS Press, Champaign, IL, 1998, pp. 232.
- [125] J. Lehrfeld, Conversion of agricultural residues into cation exchange materials, *J. Appl. Polym. Sci.* 61 (1996) 2099–2105.
- [126] M.N. Khan, M.F. Wahab, Characterization of chemically modified corncobs and its application in the removal of metal ions from aqueous solution, *J. Hazard. Mater. B* 141 (2006) 237–244.
- [127] L.A. Leyva Ramos, R. Bernal Jacome, I. Acosta Rodriguez, Adsorption of cadmium(II) from aqueous solution on natural and oxidized corncob, *Sep. Purif. Technol.* 45 (2005) 41–49.
- [128] L. Billon, V. Meric, A. Castetbon, J. Francois, J. Desbrieres, Removal of copper ions from water of boilers by a modified natural based, corncobs, *J. Appl. Polym. Sci.* 102 (2006) 4637–4645.
- [129] W.E. Marshall, M.M. Johns, Agricultural by products as metal adsorbents: sorption properties and resistance to mechanical abrasion, *J. Chem. Technol. Biotechnol.* 66 (1996) 192–198.
- [130] R.E. Wing, Corn fiber citrate: preparation and ion exchange properties, *Ind. Crops Prod.* 5 (1996) 301–305.
- [131] L.H. Wartelle, W.E. Marshall, Citric acid modified agricultural by products as copper ion adsorbents, *Adv. Environ. Res.* 4 (2000) 1–7.
- [132] R. Gong, Y. Jin, F. Chen, J. Chen, Z. Liu, Enhanced malachite green removal from aqueous solution by citric acid modified rice straw, *J. Hazard. Mater.* 137 (2006) 865–870.
- [133] W.E. Marshall, L.H. Wartelle, Acid recycling to optimize citric acid modified soybean hull production, *Ind. Crops Prod.* 18 (2003) 177–182.
- [134] FAO (Food and Agriculture Organization of The United Nations), <http://www.fao.org/docrep/004/ad452e/ad452e1i.htm#TopOfPage>, (2003).
- [135] C.L. Chuang, M. Fan, M. Xu, R.C. Brown, S. Sung, B. Saha, C.P. Huang, Adsorption of arsenic(V) by activated carbon prepared from oat hulls, *Chemosphere* 61 (2005) 478–483.

# Modification of Straw for Activated Carbon Preparation and Application for the Removal of Dyes from Aqueous Solutions

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## 7.3.1 INTRODUCTION

Many industries, such as dyestuffs, textile, paper, plastics, tannery, and paint use dyes to color their products and also consume substantial volumes of water. As a result, they generate a considerable amount of colored wastewater [1]. The presence of very small amounts of dyes in water (less than 1 ppm for some dyes) is highly visible and undesirable [2, 3]. According to Chakrabarti et al. [4], nearly 40 000 dyes and pigments are listed, which consist of more than 7000 different chemical structures. Most of them are completely resistant to biodegradation processes [5]. Over 100 000 commercially available dyes exist and more than  $7 \times 10^5$  tons are produced worldwide annually [6, 7]. Recent studies indicate that approximately 12% of produced synthetic dyes are lost during manufacturing and processing operations. Approximately 20% of these lost dyes enter the industrial wastewaters [8, 9]. An indication of the scale of the problem is given by the fact that 10–15% of the dye is lost in the effluent during the dyeing process [10, 11].

Dye molecules consists of two key components: the chromophores, which are largely responsible for producing the color, and the auxochromes, which not only supplement the chromophore but also render the molecule soluble in water and enhance its affinity (to attach) toward the fibers [12]. Dyes may be classified in several ways, according to chemical constitution, application class, and end use. Dyes are here classified according to how they are used in the dyeing process. Main dyes are grouped as acid dyes, basic dyes, direct dyes, mordant dyes, vat dyes, reactive dyes, disperse dyes, azo dyes, and sulfur dyes. Typical dyes used in textile dyeing operations are given in Table 7.3.1 [13].

As a result of increasingly stringent restrictions on the organic content of industrial effluents, it is necessary to eliminate dyes from wastewater before it is discharged. Many of these dyes are also toxic and even carcinogenic [14, 15]. Besides this, they also interfere with the transmission of light and upset the biological metabolism processes, which causes the destruction of aquatic communities present in various ecosystems [16, 17]. Furthermore, the dyes have a tendency to sequester metal and may cause microtoxicity to fish and other organisms [17]. However, wastewater containing dyes is very difficult to treat because the dyes are recalcitrant organic molecules, which are resistant to aerobic digestion, and are stable to light, heat, and oxidizing agents [18, 19]. So color is the first contaminant to be recognized in the wastewater [3].

## 7.3.2 TECHNOLOGIES AVAILABLE FOR DYE REMOVAL

Methods of dye wastewater treatment have been reviewed recently [2, 3, 20–23]. There are several reported treatment methods for the removal of dyes from effluents and these technologies can be divided into three categories: biological methods, chemical methods, and physical methods [2]. However, all of them have advantages and drawbacks. Because of the high cost and disposal problems, many of these conventional methods for treating dye wastewater have not been widely applied at large scale in the textile and paper industries [24]. At the present time, there is no single process capable of adequate treatment, mainly because of the complex nature of the effluents [25, 26].

**TABLE 7.3.1** Typical Dyes Used in Textile Dyeing Operations

Dye class	Description
Acid	Water soluble anionic compounds
Basic	Water soluble, applied in weakly acidic dyebaths; very bright dyes
Direct	Water soluble, anionic compounds; can be applied directly to cellulose without mordants (or metals like chromium and copper)
Disperse	Not water soluble
Reactive	Water soluble, anionic compounds; largest dye class
Sulfur	Organic compounds containing sulfur or sodium sulfide
Vat	Water insoluble; oldest dyes; more chemically complex

### 7.3.2.1 Biological Treatments

Biological treatment is the most common and widespread technique used in dye wastewater treatment [27–32], and it is often the most economical alternative when compared with other physical and chemical processes. The process can be either aerobic (in the presence of oxygen), anaerobic (without oxygen), or combined aerobic/anaerobic [12].

Bacteria and fungi are the two microorganism groups that have been most widely studied for their ability to treat dye wastewaters, which have been reviewed by Aksu [33], Wesenberg et al. [34], Pearce et al. [6], McMullan et al. [7], Fu and Viraraghavan [35], and Stolz [36]. A number of triphenylmethane dyes, such as magenta, crystal violet, pararosaniline, brilliant green, malachite green (MG), and ethyl violet, have been found to be efficiently decolorized (92–100%) by the strain *Kurthia* sp. [37]. Generally, the factors such as concentration of dyes, initial pH, and temperature of the effluent, affect the decolorization process. Although this methodology is cost-competitive, and biological treatments are suitable for a variety of dyes, the main drawbacks of the biological treatment is low biodegradability of the dyes, less flexibility in design and operation, larger land area requirement, and longer times required for the decolorization/fermentation processes, thereby making it incapable of removing dyes from effluent on a continuous basis in liquid-state fermentations [2, 38, 39].

### 7.3.2.2 Chemical Methods

Chemical methods include coagulation or flocculation combined with flotation and filtration, precipitation/flocculation with  $\text{Fe(II)/Ca(OH)}_2$ , conventional oxidation methods by using oxidizing agents (chemical oxidation and ultraviolet [UV]-assisted oxidation using chlorine, hydrogen peroxide, Fenton's reagent, ozone, or potassium permanganate), electrochemical methodology [1, 40, 41] by electro-

oxidation with nonsoluble anodes or by electro-coagulation using consumable materials, and advanced oxidation processes, which are the processes involving simultaneous use of more than one oxidation processes, since sometimes a single oxidation system is not sufficient for the total decomposition of dyes. The key to advanced oxidation processes is that all of these reactions involve the accelerated production of the hydroxyl free radical, which is very reactive. The techniques include Fenton's reagent oxidation, UV photolysis, and sonolysis. Although the dyes are removed, these chemical techniques are often expensive. The high demand for electrical energy and the consumption of chemical reagents are common problems. And the accumulation of concentrated sludge creates a disposal problem. There is also the possibility that a secondary pollution problem will arise because of excessive chemical use.

### 7.3.2.3 Physical Methods

Different physical methods are also widely used, such as membrane-filtration processes (ultrafiltration, nanofiltration, reverse osmosis, electrodialysis) and adsorption techniques. Reverse osmosis [42–44] is effective decoloring and desalting process against the most diverse range of dye wastes, and it has been successfully used for recycling. The water produced by reverse osmosis, like distilled water, will be close to pure  $\text{H}_2\text{O}$ . The main drawbacks of the membrane processes are high working pressures, significant energy consumption, high cost of membrane and a relatively short membrane life, which limits their use for treating the dye wastewater.

In accordance with the very abundant literature data, adsorption has been proved to be an excellent way to treat industrial waste effluents, offering significant advantages like the low cost, availability, profitability, ease of operation and efficiency, in comparison with the conventional methods, especially from economical and environmental



points of view [45–47]. This process provides an attractive alternative for the treatment of contaminated waters, especially if the sorbent is inexpensive and does not require an additional pretreatment step before its application.

### 7.3.3 DYE REMOVAL USING COMMERCIAL ACTIVATED CARBONS

Adsorption methods employing solid sorbents are widely used to remove certain classes of chemical pollutants from wastewater. However, among all the sorbent materials proposed, activated carbon (AC) is the most popular material for the removal of pollutants from wastewater. AC is an amorphous carbon in which a high degree of porosity has been developed during manufacture. It is this porosity, which governs the way in which AC performs its purifying role, and the very large surface area provides many sites upon which the adsorption of impurity molecules can take place [48]. In particular, the effectiveness of adsorption on commercial activated carbons for the removal of a wide variety of dyes from wastewaters has made it an ideal alternative to other expensive treatment options [49]. As we know, AC is usually prepared from coal, coconut shells, lignite, wood, straw, etc., using one of the two basic activation methods: physical activation and chemical activation [50–54]. Generally, the physical activation requires high temperature and longer activation time as compared with chemical activation. However, in chemical activation, the AC needs a thorough washing because of the use of chemical agents. The product formed by either of the methods is known as AC and normally has a very porous structure with a large surface area ranging from 500 to 2000 m<sup>2</sup>·g<sup>-1</sup> [55].

AC is available in two main forms: powdered activated carbon (PAC) and granular activated carbon (GAC). Most of the work on the removal of pollutants from water has been on GAC because of the fact that the granular form is more adaptable to continuous contacting and there is no need to separate the carbon from the bulk fluid. However, the use of PAC presents some practical problems because of the requirement to separate the adsorbent from the fluid after use. In spite of these problems, however, PAC is also used for wastewater treatment because of low capital cost and lesser contact time requirement [56]. Besides PAC and GAC, two other forms of ACs are also available, activated carbon pellet (ACP) and activated carbon fiber (ACF). The pelletized ACs, generally, are prepared from coal where coal is pulverized and reagglomerated with suitable binder and then physically activated. For ACF, the carbon fibers are generally prepared from polymeric precursor materials such as polyacrylonitrile, cellulose, pitch, and polyvinylchloride [12].

To demonstrate the versatility of commercial AC, this adsorbent for different types of dyes has been applied in published articles [57–60]. McKay [61] used AC of Filtrasorb type for the removal of acidic, basic, disperse, and direct dyes, and found it to be excellent for the removal of all except the direct dyes. The adsorption of three reactive dyes used in textile industry on Filtrasorb 400 AC was studied by Al-Degs et al. [57] and some workers [58] further studied various ACs for the removal of cationic dye (methylene blue) and anionic dye (reactive black) and reported that there exists a good relationship between performance of ACs and methylene blue capacity/surface area. El Qada et al. [62] comparatively studied the adsorption capacity of three different types of ACs (PAC1, PAC2, and F400), which were produced by steam activation in small laboratory scale and large industrial scale processes. It was found that PAC2 has the highest adsorptive capacity toward methylene blue (588 mg·g<sup>-1</sup>) followed by F400 (476 mg·g<sup>-1</sup>) and PAC1 (380 mg·g<sup>-1</sup>). Basic red and basic yellow showed higher adsorptive affinity toward PAC2 and F400 than methylene blue. Adsorbent surface chemistry, surface area, pore structure, adsorbent particle size and solution pH proved to be important factors in governing the adsorption process. Karaca et al. [63] investigated the adsorption mechanism of methylene blue onto a commercial PAC from aqueous solution as a function of initial dye concentration, temperature, pH, and stirring speed. The adsorbed amount methylene blue dye on AC slightly changed with increasing pH and temperature, indicating an endothermic process. The adsorption capacity of methylene blue did not significantly change with increasing stirring speed. Adsorption measurements showed that the process was very fast and physical in nature. The adsorption behavior of C.I. Reactive Blue 2, C.I. Reactive Red 4, and C.I. Reactive Yellow 2 from aqueous solution onto a commercial AC was investigated at various experimental conditions by Al-Degs et al. [64]. The adsorption capacity of AC for reactive dyes was found to be relatively high. At pH 7.0 and 298 K, the maximum adsorption capacity for the dyes C.I. Reactive Blue 2, C.I. Reactive Yellow 2, and C.I. Reactive Red 4 was found to be 0.27, 0.24, and 0.11 mmol·g<sup>-1</sup>, respectively. Experimental data indicated that the adsorption capacity of AC for the dyes was higher in acidic rather than in basic solutions and, furthermore, revealed that the removal of dye increased with increase in the ionic strength of solution, which was attributed to the aggregation of reactive dyes in the solution. The adsorption of three acid dyes, acid red 97, acid orange 61, and acid brown 425 onto a commercial AC was studied for the removal of acid dyes from aqueous solutions at room temperature (25 °C) [65]. When a comparative study was made of the results obtained with single and mixed dyes, it can be seen that some of them affect others and modify their behavior in the adsorption

process. The results show that AC could be employed for the removal of dyes from wastewater.

Studies have shown that, in general, ACs are good materials for the removal of different types of dyes, but their use is sometimes restricted in view of higher cost. The ACs after adsorption become exhausted and are no longer capable of further adsorbing the dyes. Once exhausted, AC has to be regenerated for further use in purifying water, and a number of methods like thermal, chemical, oxidation, electrochemical [66–74] are applied for this purpose. In general, the most common is the thermal method. The regeneration of saturated carbon is also expensive, results in loss of the adsorbent, and the regenerated product may have a slightly lower adsorption capacity in comparison with the virgin AC. Furthermore, the use of carbons based on relatively expensive starting materials is also unjustified for most pollution control applications [75]. This has resulted in attempts by various workers to prepare low-cost alternative adsorbents [76], which may replace ACs in pollution control through adsorption process.

### 7.3.4 DYE REMOVAL USING AGRICULTURAL WASTES OR BYPRODUCTS

The agricultural wastes or byproducts such as cereal straws can be assumed to be low-cost adsorbents as they are abundant in nature, inexpensive, require little processing, and are effective materials. These materials are available in large quantities and have the potential as adsorbents because of their physicochemical characteristics and low cost. Many of these materials have been investigated as adsorbents for the removal of dyes, which are summarized as below.

Sawdust is an abundant byproduct of the wood industry that is either used as cooking fuel or as packing material. It is easily available in the countryside at zero or negligible price [77]. Sawdust consists of lignin, cellulose, and hemicelluloses, with polyphenolic groups playing an important role for binding dyes through different mechanisms. Generally, the adsorption takes place by complexation, ion exchange, and hydrogen bonding. Various fruitful studies have been done on the removal of dyes using sawdust [78, 79], and some adsorption capacities are reported in Table 7.3.2. One problem with sawdust materials is that the results of sorption are strongly pH-dependent [96, 97, 104, 105]. In a study on the removal of acid and basic dyes by sawdust, Ho and McKay [97] reported that the sorption capacity of basic dye (basic blue 69) was much higher than acid dye (acid blue 25), which was because of the ionic charges on the dyes and the character of the sawdust. Similar to other materials, the adsorption capacity of sawdust can also be improved by using chemical treatment [96, 105, 106].

Bark, a polyphenol-rich material, is an abundant forest residue which has been found to be effective in removing

dyes from water solutions. Because of its low cost and high availability, bark is very attractive as an adsorbent. Its high tannin content is the reason for the bark's effective adsorbent capacity [94, 107]. The polyhydroxy polyphenol groups of tannin are thought to be the active species in the adsorption process. Rice husk as obtained from a local rice mill grounded, sieved, washed, and then dried at 80 °C was used by McKay et al. [80] for the removal of two basic dyes, safranin and methylene blue, and adsorption capacity of 838 and 312 mg·g<sup>-1</sup>, respectively, was found. Barley husk and corncob have been studied by Robinson et al. [108] for their effectiveness in the removal of dyes from an artificial effluent containing five dyes. It was found that 1 g (per 100 ml) of ≤600 μm corncob was found to be effective in removing a high percentage of dyes at a rapid rate (92% in 48 h), whereas 1 g of 1 × 4 mm barley husk was found to be the most effective weight and particle size combination for the removal of dyes (92% in 48 h). The results illustrate how barley husk and corncob are effective biosorbents concerning the removal of textile dyes from the effluent.

Recently, few investigations have been reported using straw for dye adsorption. Nigam et al. [109] found that up to 70–75% color removal was achieved from 500 ppm dye solutions at room temperature using wheat straw. To enhance the adsorbent capacity of straw more attention had been paid to chemical modification of the raw material. Gong et al. [110] studied the dye adsorption onto citric acid esterifying wheat straw (EWS). The results showed that the adsorption percentages of methylene blue and crystal violet maintained more than 95% over a range from 50 to 350 mg·L<sup>-1</sup> of dye concentration when 2.0 g·L<sup>-1</sup> of EWS was used. The isothermal data followed the Langmuir model. The maximum adsorption capacity ( $q_m$ ) of EWS for methylene blue and crystal violet was 312.50 and 227.27 mg·g<sup>-1</sup>, respectively. Besides, the dye adsorption of modified rice straw was also studied by Gong and coworkers [111, 112]. Rice straw was modified in two different ways, esterification with oxalic acid and phosphorylation with phosphoric acid. Then, the modified rice straw was further loaded with sodium ion for enhancing its cationic sorption capacity. The results illustrated that the sorption capacities of esterified rice straw for basic blue 9 and basic green 4 were 256.4 and 238.1 mg·g<sup>-1</sup>, whereas the sorption capacities of phosphorylated rice straw for basic blue 9 and basic red 5 were 208.33 and 188.68 mg·g<sup>-1</sup>, respectively. In both conditions, increase in ion strength of solution induced decline of dyes sorption. Barley straw was modified by a surfactant, cetylpyridinium chloride, and used as an adsorbent for acid blue 40 and reactive black 5 [113]. It was found that the surfactant-modified barley straw exhibits higher adsorption to acid blue 40 than reactive black 5, and the adsorption of the dyes is influenced by several parameters such as initial concentration of the dye, adsorbent dosage, solution pH, and adsorption temperature. Adsorption isotherms show that maximum adsorption of acid blue 40

**TABLE 7.3.2** Recent Reported Adsorption Capacities  $q_m$  (mg·g<sup>-1</sup>) for Agricultural Wastes or Byproducts

Adsorbent	Dye	$q_m$	Ref.
Bark	Basic red 2	1119	[80]
Bark	Basic blue 9	914	[80]
Rice husk	Basic red 2	838	[80]
Tree fern	Basic red 13	408	[81]
Pine sawdust	Acid yellow 132	398.8	[79]
Palm fruit bunch	Basic yellow	327	[82]
Sunflower stalk	Basic red 9	317	[83]
Rice husk	Basic blue 9	312	[80]
Spent tea leaves	Basic blue 9	300.05	[84]
Jack fruit peel	Basic blue 9	285.71	[85]
Pine sawdust	Acid blue 256	280.3	[79]
Vine	Basic red 22	210	[86]
Sunflower stalk	Basic blue 9	205	[83]
Coir pith	Basic violet 10	203.25	[87]
Soy meal hull	Direct red 80	178.57	[88]
Rice hull ash	Direct red 28	171	[89]
Egyptain bagasse pith	Basic blue 69	168	[90]
Vine	Basic yellow 21	160	[86]
Bagasse pith	Basic blue 69	157.4	[80]
Egyptain bagasse pith	Basic blue 69	152	[91]
Cedar sawdust	Basic blue 9	142.36	[92]
Soy meal hull	Direct red 81	120.48	[88]
Coir pith	Basic blue 9	120.43	[93]
Soy meal hull	Acid blue 92	114.94	[88]
Soy meal hull	Acid red 14	109.89	[88]
Coir pith	Basic violet 10	94.73	[93]
Palm fruit bunch	Basic blue 3	92	[82]
Eucalyptus bark	Remazol BB	90	[94]
Raw date pits	Raw date pits	80.3	[46]
Hazelnut shell	Basic blue 9	76.9	[95]
Bagasse pith	Basic red 22	76.6	[80]
Egyptian bagasse pith	Basic red 22	75	[91]
Treated sawdust	Basic green 4	74.5	[96]
Wood sawdust	Basic blue 69	74.4	[97]
Palm oil ash	Disperse red	61.35	[98]
Palm oil ash	Disperse blue	49.5	[98]

(Continued)

**TABLE 7.3.2** Recent Reported Adsorption Capacities  $q_m$  (mg·g<sup>-1</sup>) for Agricultural Wastes or Byproducts—cont'd

Adsorbent	Dye	$q_m$	Ref.
Maize cob	Acid red 114	47.7	[99]
Maize cob	Acid blue 25	41.4	[99]
Sunflower stalk	Direct red 28	37.78	[83]
Treated sawdust	Basic green 4	26.9	[96]
Sunflower stalk	Direct blue	26.84	[83]
Banana peel	Methyl orange	21	[100]
Banana peel	Basic blue 9	20.8	[100]
Banana peel	Basic violet 10	20.6	[100]
Orange peel	Methyl orange	20.5	[100]
Egyptian bagasse pith	Acid red 114	20	[91]
Orange peel	Acid violet	19.88	[101]
Orange peel	Basic blue 9	18.6	[100]
Banana peel	Direct red 28	18.2	[100]
Egyptian bagasse pith	Acid blue 25	17.5	[91]
Egyptian bagasse pith	Acid blue 25	14.4	[90]
Orange peel	Basic violet 10	14.3	[100]
Coir pith	Acid violet	7.34	[87]
Wood sawdust	Acid blue 25	5.99	[97]
Banana pith	Direct red	5.92	[102]
Sugar cane dust	Basic green 4	4.88	[103]
Neem sawdust	Basic violet 3	3.78	[104]
Neem sawdust	Basic green 4	3.42	[104]

and reactive black 5 is  $1.02 \times 10^{-4}$  and  $2.54 \times 10^{-5}$  mol·g<sup>-1</sup>, respectively. It should be mentioned that the initial dye concentration, contact time, sorbent dosage, and pH are the most effective parameters for adsorption of dyes. The effects of these parameters on the adsorption of dyes can be examined by optimal experimental conditions.

Other agricultural wastes or byproducts from cheap and readily available resources such as tree fern, palm-fruit bunch, sunflower stalks, coir pith, bagasse pith, orange peel, corncob, and rice hull ash have also been successfully used for the removal of dyes from aqueous solution (Table 7.3.2).

### 7.3.5 PRODUCTION OF ACS FROM AGRICULTURAL BYPRODUCTS

Any cheap material, with a high carbon content and low inorganics, can be used as a raw material for the production

of AC [114]. Agricultural byproducts are available in large quantities and are one of the most abundant renewable resources in the world. These waste materials have little or no economic value and often present a disposal problem. Therefore, there is a need to valorize these low cost byproducts. Thus, conversion of waste materials into ACs would add considerable economic value, help reduce the cost of waste disposal and most importantly provide a potentially inexpensive alternative to the existing commercial ACs. These waste materials have proved to be promising raw materials for the production of AC with a high adsorption capacity, considerable mechanical strength, and low ash content [115]. Because of their low cost, after being expended, these materials can be disposed of without expensive regeneration. A wide variety of ACs have been prepared from agricultural byproducts such as corn straw [116], wheat straw [116], rice straw [117, 118], sawdust [77, 119–121], corn cob [122], bagasse [122, 123], cotton stalk

[124], coconut husk [125], rice husks [121, 126, 127], tobacco stem [128], nut shells [129, 130], soybean oil cake [131], oil palm shell [132], and oil palm fiber [133].

Basically, there are two main steps for the preparation and manufacture of AC: (1) the carbonization of the carbonaceous raw material below 1000 °C, in an inert atmosphere, and (2) the activation of the carbonized product (char), which is either physical or chemical.

### 7.3.5.1 Physical Activation

Physical activation is a process in which the precursor is developed into AC using gases and is generally carried out in a two-step process. Carbonization is the first stage and involves the formation of a char, which is normally nonporous, by pyrolysis of the precursor at temperatures in the range between 400 and 850 °C, and sometimes reaches 1000 °C, in an inert, usually nitrogen, atmosphere. Activation is the second stage and involves contacting the char with an oxidizing gas, such as carbon dioxide, steam, air or their mixtures, in the temperature range between 600 and 900 °C, which results in the removal of the more disorganized carbon and the formation of a well-developed micropore structure. The activation gas is usually CO<sub>2</sub>, since it is clean, easy to handle and it facilitates control of the activation process because of the slow reaction rate at temperatures around 800 °C [134]. It is worthwhile noting that the ACs produced by physical activation did not have satisfactory characteristics to be used as adsorbents or as filters [135].

### 7.3.5.2 Chemical Activation

Chemical activation involves impregnation with chemicals such as ZnCl<sub>2</sub>, KOH, NaOH, H<sub>3</sub>PO<sub>4</sub>, or K<sub>2</sub>CO<sub>3</sub> followed by heating under a nitrogen flow at temperatures in the range of 450–900 °C, depending on the impregnant used. In the chemical activation process, carbonization and activation are carried out simultaneously, with the precursor being mixed with chemical activating agents, such as dehydrating agents and oxidants. Chemical activation offers several advantages as it is carried out in a single step, performed at lower temperatures and, therefore, resulting in the development of a better porous structure, although the environmental concerns of using chemical agents for activation could be developed. Besides, part of the added chemicals (such as zinc salts and phosphoric acid), can be easily recovered [114, 134, 136]. However, a two-step process (an admixed method of physical and chemical processes) can be applied [118].

It is to be noted that there is also an additional one-step treatment route, denoted as steam-pyrolysis as reported [115, 137–141], where the raw agricultural residue is either heated at moderate temperatures (500–700 °C) under a flow of pure steam, or heated at 700–800 °C under a flow of steam. Many agricultural residues such as straw,

bagasse, apricot stones, cherry stones, grape seeds, nutshells, almond shells, oat hulls, corn stover, and peanut hulls have been studied with this method. AC produced with this method will not be discussed here, but more details are available [135].

### 7.3.5.3 Dye Removal Using ACs from Agricultural Byproducts

The ability of ACs prepared from agricultural byproducts such as cereal straws as sorbents to remove various dyes from aqueous solutions and wastewaters were extensively investigated. Valix et al. [123] showed that despite the high ash content of bagasse, high surface areas (614–1433 m<sup>2</sup>·g<sup>-1</sup>) and microporous (median pore size from 0.45 to 1.2 nm) ACs can be generated through a low-temperature (160 °C) chemical (concentrated sulfuric acid) carbonization treatment and gasification with carbon dioxide at 900 °C. The micropore area of the AC developed from chars prepared by this method provides favorable adsorption sites to acid blue 80 (391 mg·g<sup>-1</sup> of carbon), which was higher than commercially available carbon (121 mg·g<sup>-1</sup> of Filtrasorb 400) despite the high ash content of the final carbon (61 wt%).

Although most carbonaceous substances can be converted into AC, the final properties of the carbon will depend significantly on the nature of the starting material. ACs were prepared by Hameed [77] and Tan [125] and their coworkers from rattan sawdust and coconut husk as the adsorbents for the removal of methylene blue dye from an aqueous solution. It was found that the BET surface area, average pore diameter and pore volume of the AC were 1083 m<sup>2</sup>·g<sup>-1</sup>, 2.77 nm, and 0.644 cm<sup>3</sup>·g<sup>-1</sup> for the former, while they were 1940 m<sup>2</sup>·g<sup>-1</sup>, 2.36 nm, and 1.143 cm<sup>3</sup>·g<sup>-1</sup> for the latter. Equilibrium data fitted well with the Langmuir model with maximum monolayer adsorption capacity of 294.14 mg·g<sup>-1</sup> and 434.78 mg·g<sup>-1</sup>, respectively. When coconut husk was used, the surface area of the prepared AC was relatively high with large pore volume, and was found to be mesoporous. Furthermore, it was revealed that methylene blue could adsorb strongly on the surface of the AC.

However, besides the different sources of raw materials the adsorption capacities of a carbon also depend on the history of its preparation and treatment conditions such as pyrolysis temperature and activation time. Prakash Kumar et al. [119] comparatively studied the adsorption of Bismark Brown dye on activated carbons prepared from rubber wood sawdust by three different activation methods including chemical activation (the char was impregnated with phosphoric acid of 0.45 impregnation ratio (IR) (g phosphorous: g precursor) and activated in fixed bed at 400 °C for 1 h), steam-activation (the char was activated in a fluidized bed reactor at 750 °C for 1 h with steam flow rate of 4 mL·min<sup>-1</sup>), and chemical followed by steam activation (the char was impregnated with phosphoric acid (0.45) IR and



activated in a fluidized bed at 800 °C for 1 h with steam flow rate of 5 mL·min<sup>-1</sup>). The produced ACs were named chemical-AC (CAC), steam-AC (SAC), and chemical followed by steam-AC (CSAC), respectively. The BET surface area were 822, 1092, and 954 m<sup>2</sup>·g<sup>-1</sup> for CAC, SAC, and CSAC, respectively. Excellent adsorption capacities of 2000 and 1111 mg·g<sup>-1</sup> were obtained for SAC and CSAC, respectively, whereas the adsorption capacity was only 164 mg·g<sup>-1</sup> for CAC. The adsorption of MG from aqueous medium by rice husk-based porous carbons, oxidized carbons, and their heat-treated derivatives were studied by Guo et al. [126]. The results illustrated that the adsorption capacity of carbons activated by NaOH-activation was larger than that of carbons activated by KOH-activation, and the adsorption of MG on oxidized carbons decreased and was enhanced after heat-treatment. It was also found that the adsorption capacity did not always increase with surface area.

Many factors can affect the adsorption capacity in the same sorption conditions such as surface chemistry, surface charge, and pore structure [25, 142–144]. The surface chemistry of ACs plays a key role in the performance of the reactive dye adsorption process. Moreover, it is possible to tailor the chemical properties of ACs, by

appropriate chemical and thermal treatments, to maximize the adsorption capacity. Tan et al. [132] investigated the effect of hydrochloric acid treatment of AC prepared from oil palm shell on methylene blue adsorption. The Fourier transform infrared spectroscopy revealed that the acid treatment influenced the surface chemistry of the AC and produced more acidic groups such as carboxylic and ether, resulting in the adsorption capacity of 303.03 mg·g<sup>-1</sup> for HCl-treated AC, which was 24.24% higher compared with the untreated AC. The adsorption capacity also depends on the accessibility of the pollutants to the inner surface of the adsorbent, which depends on their size [145]. Chan et al. [146] reported that the high surface area AC produced by thermal activation of bamboo with phosphoric acid showed nearly three times higher adsorption capacity for small dye molecule, acid blue 25, than the commercial carbon (F400) and it has similar capacity as F400 for larger size dye acid yellow 117. Both surface area and porosity of the carbon have played an important role in the adsorption of the dyes.

Many other agricultural byproducts-based ACs were manufactured using different methods, which exhibited high sorption properties as shown in Table 7.3.3.

**TABLE 7.3.3** Recent Reported Adsorption Capacities  $q_m$  (mg·g<sup>-1</sup>) for ACs Made from Agricultural Byproducts

Raw material	Dye	$q_m$	Sources
Rubberwood sawdust	Bismark Brown	2000	[119]
Rubberwood sawdust	Bismark Brown	1111	[119]
Corn cob	Acid blue 25	1060	[122]
Bagasse	Basic red 22	942	[122]
Cane pith	Basic red 22	941.7	[147]
Palm kernel	Acid blue 25	904	[122]
Corn cob	Basic red 22	790	[122]
Palm kernel	Basic red 22	710	[122]
Bagasse	Acid blue 25	674	[122]
Cane pith	Acid blue 25	673.6	[147]
Rice husk	Basic green 4	511	[126]
Straw	Basic blue 9	472.1	[148]
Coconut husk	Basic blue 9	434.78	[125]
Bagasse	Acid blue 80	391	[123]
Pine sawdust	Basic green 4	370.37	[149]
Rice husk	Basic blue 9	343.5	[148]
Palm kernel shell	Basic blue 9	311.72	[150]
Oil palm shell (HCl treated)	Basic blue 9	303.03	[132]

(Continued)

Raw material	Dye	$q_m$	Sources
Rattan sawdust	Basic blue 9	294.14	[77]
Coconut shell	Basic blue 9	277.9	[148]
Oil palm fiber	Basic blue 9	277.78	[133]
Oil palm shell	Basic blue 9	243.90	[151]
Rubber seed coat	Basic blue 3	227.27	[152]
Groundnut shell	Basic green 4	222.22	[153]
Pongam seed shell	Acid red 114	204.08	[154]
Jute fiber	Reactive red 120	200	[155]
Mahogany sawdust	Acid yellow 36	183.8	[121]
Coconut tree flower	Reactive red 120	181.9	[155]
Apricot stone	Astrazon blue FGRL	181.5	[156]
Waste apricot	Malachite green	157	[157]
Cotton seed shell	Acid red 114	153.85	[154]
Bamboo dust	Basic blue 9	143.2	[148]
Waste apricot	Basic blue 9	140	[157]
Orange peel	Direct navy blue 106	107.53	[158]
Waste apricot	Crystal violet	89	[157]
Rice husk	Acid yellow 36	86.9	[121]
Sawdust	Basic green 4	74.5	[96]
Rice husk	Acid blue	50	[127]
<i>Rosa canina</i> sp. seeds	Basic blue 9	47.2	[159]
Rice husk	Basic blue 9	19.83	[148]
Straw	Basic blue 9	19.82	[148]
Date pits	Basic blue 9	17.3	[46]
Coir pith	Acid violet	8.06	[87]
Coir pith	Direct red 28	6.72	[160]
Sugarcane bagasse	Acid orange 10	5.78	[161]
Coir pith	Basic violet 10	2.56	[87]

### 7.3.6 SUMMARY

The agricultural byproducts such as cereal straws can be used as adsorbents with little or no pretreatment and can, therefore, be manufactured at low cost for dye removal. To enhance the adsorption capacity, agricultural byproducts could be manufactured to ACs in many different methods. In both cases, it would add considerable economic value,

help reduce the cost of waste disposal and most importantly provide a potentially inexpensive alternative to the existing commercial ACs. In a word, the prospect of using low-cost materials available mostly from agricultural byproducts provides a route to cleaner, cheaper, and efficient technology. However, adsorption is a complicated process depending on several interactions such as electrostatic and nonelectrostatic (hydrophobic) interactions, and the specific

sorption mechanisms by which the adsorption of dyes takes place on these adsorbents are still not clear. Although much has been accomplished in terms of sorption properties and kinetics, more work is still necessary to identify the sorption mechanisms clearly. In addition, since the dye effluents contain several other pollutants, attention has to be given to adsorption of dyes from mixtures, and the adsorbents based on cereal straws should be further investigated for their efficiency by using dye effluents from industries. At last, to promote their large-scale use, efforts are needed to carry out a cost comparison between conventional and agricultural byproducts based adsorbents.

## REFERENCES

- [1] C. Grégorio, Non conventional low cost adsorbents for dye removal: a review, *Bioresour. Technol.* 97 (2006) 1061–1085.
- [2] T. Robinson, G. McMullan, R. Marchant, P. Nigam, Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative, *Bioresour. Technol.* 77 (2001) 247–255.
- [3] I.M. Banat, P. Nigam, D. Singh, R. Marchant, Microbial decolorization of textile dye containing effluents: a review, *Bioresour. Technol.* 58 (1996) 217–227.
- [4] T. Chakrabarti, P.V.R. Subrahmanyam, B.B. Sundaresan, Biodegradation of recalcitrant industrial wastes, in: D.L. Wise (Ed.), *Biotreatment Systems*, CRC Press, 1988, pp. 171–234.
- [5] P. Pitter, J. Chudoba, *Biodegradability of Organic Substances in the Aquatic Environment*, CRC Press, Inc., Boca Raton, 1990.
- [6] C.I. Pearce, J.R. Lloyd, J.T. Guthrie, The removal of colour from textile wastewater using whole bacterial cells: a review, *Dyes Pigm.* 58 (2003) 179–196.
- [7] G. McMullan, C. Meehan, A. Conneely, N. Kirby, T. Robinson, P. Nigam, I.M. Banat, R. Marchant, W.F. Smyth, Microbial decolourisation and degradation of textile dyes, *Appl. Microbiol. Biotechnol.* 56 (2001) 81–87.
- [8] M. Hema, S. Arivoli, Comparative study on the adsorption kinetics and thermodynamics of dyes onto acid activated low cost carbon, *Int. J. Phys. Sci.* 2 (2007) 10–17.
- [9] A.A. Essawy, A. El Hag Ali, M.S.A. Abdel Mottaleb, Application of novel copolymer TiO<sub>2</sub> membranes for some textile dyes adsorptive removal from aqueous solution and photocatalytic decolorization, *J. Hazard. Mater.* 157 (2008) 547–552.
- [10] L. Young, Y.U. Jain, Ligninase catalyzed decolorization, *Water Res.* 31 (1997) 1187–1193.
- [11] L. Lian, L. Guo, C. Guo, Adsorption of Congo red from aqueous solutions onto Ca bentonite, *J. Hazard. Mater.* 161 (2009) 126–131.
- [12] V.K. Gupta, Suhas, Application of low cost adsorbents for dye removal – a review, *J. Environ. Manage.* 90 (2009) 2313–2342.
- [13] D. Ayhan, Agricultural based activated carbons for the removal of dyes from aqueous solutions: a review, *J. Hazard. Mater.* (2009) doi:10.1016/j.jhazmat.2008.12.114.
- [14] C. O'Neill, F.R. Hawkes, D.L. Hawkes, N.D. Lourenço, H.M. Pinheiro, W. Delée, Colour in textile effluents – sources, measurement, discharge consents and simulation: a review, *J. Chem. Technol. Biotechnol.* 74 (1999) 1009–1018.
- [15] P.C. Vandevivere, R. Bianchi, W. Verstraete, Treatment and reuse of wastewater from the textile wet processing industry: review of emerging technologies, *J. Chem. Technol. Biotechnol.* 72 (1998) 289–302.
- [16] W.G. Kuo, Decolorizing dye waste water with fenton reagent, *Water Res.* 26 (1992) 881–886.
- [17] G.E. Walsh, L.H. Bahner, W.B. Horning, Toxicity of textile mill effluents to freshwater and estuarine algae, crustaceans and fishes, *Environ. Pollut.* 21 (1980) 169–179.
- [18] Q. Sun, L. Yang, The adsorption of basic dyes from aqueous solution on modified peat resin particle, *Water Res.* 37 (2003) 1535–1544.
- [19] M.N.V. Ravi Kumar, T.R. Sridhari, K.D. Bhavani, P.K. Dutta, Trends in color removal from textile mill effluents, *Colorage* 40 (1998) 25–34.
- [20] D. Pokhrel, T. Viraraghavan, Treatment of pulp and paper mill wastewater – a review, *Sci. Total Environ.* 333 (2004) 37–58.
- [21] Y.M. Slokar, A. Majcen Le Marechal, Methods of decoloration of textile wastewaters, *Dyes Pigm.* 37 (1998) 335–356.
- [22] W. Delée, C. O'Neill, F.R. Hawkes, H.M. Pinheiro, Anaerobic treatment of textile effluents: a review, *J. Chem. Technol. Biotechnol.* 73 (1998) 323–335.
- [23] P. Cooper, Removing color from dyehouse waste waters: a critical review of technology available, *J. Soc. Dyers Colour.* 109 (1993) 97–100.
- [24] S.M. Ghoreishi, R. Haghighi, Chemical catalytic reaction and biological oxidation for treatment of non biodegradable textile effluent, *Chem. Eng. J.* 95 (2003) 163–169.
- [25] M.F.R. Pereira, S.F. Soares, J.J.M. Órfão, J.L. Figueiredo, Adsorption of dyes on activated carbons: influence of surface chemical groups, *Carbon* 41 (2003) 811–821.
- [26] A. Marco, S. Esplugas, G. Saum, How and why to combine chemical and biological processes for wastewater treatment, *Water Sci. Technol.* 35 (1997) 231–237.
- [27] B.E. Barragan, C. Costa, M. Carmen Marquez, Biodegradation of azo dyes by bacteria inoculated on solid media, *Dyes Pigm.* 75 (2007) 73–81.
- [28] K.C.A. Bromley Challenor, J.S. Knapp, Z. Zhang, N.C.C. Gray, M.J. Hetheridge, M.R. Evans, Decolorization of an azo dye by unacclimated activated sludge under anaerobic conditions, *Water Res.* 34 (2000) 4410–4418.
- [29] A.B. dos Santos, F.J. Cervantes, J.B. van Lier, Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology, *Bioresour. Technol.* 98 (2007) 2369–2385.
- [30] C.T.M.J. Frijters, R.H. Vos, G. Scheffer, R. Mulder, Decolorizing and detoxifying textile wastewater, containing both soluble and insoluble dyes, in a full scale combined anaerobic/aerobic system, *Water Res.* 40 (2006) 1249–1257.
- [31] F.P. van der Zee, S. Villaverde, Combined anaerobic aerobic treatment of azo dyes – a short review of bioreactor studies, *Water Res.* 39 (2005) 1425–1440.
- [32] F.M. Zhang, J.S. Knapp, K.N. Tapley, Decolourisation of cotton bleaching effluent in a continuous fluidized bed bioreactor using wood rotting fungus, *Biotechnol. Lett.* 20 (1998) 717–723.
- [33] Z. Aksu, Application of biosorption for the removal of organic pollutants: a review, *Process Biochem.* 40 (2005) 997–1026.
- [34] D. Wesenberg, I. Kyriakides, S.N. Agathos, White rot fungi and their enzymes for the treatment of industrial dye effluents, *Bio technol. Adv.* 22 (2003) 161–187.

- [35] Y.Z. Fu, T. Viraraghavan, Fungal decolorization of dye wastewaters: a review, *Bioresour. Technol.* 79 (2001) 251–262.
- [36] A. Stolz, Basic and applied aspects in the microbial degradation of azo dyes, *Appl. Microbiol. Biotechnol.* 56 (2001) 69–80.
- [37] R. Sani, U. Banerjee, Decolorization of triphenylmethane dyes and textile and dye stuff effluent by *Kurthia* sp, *Enzyme Microb. Technol.* 24 (1999) 433–437.
- [38] K.G. Bhattacharyya, A. Sarma, Adsorption characteristics of the dye, Brilliant Green, on Neem leaf powder, *Dyes Pigm.* 57 (2003) 211–222.
- [39] G. Crini, Non conventional low cost adsorbents for dye removal: a review, *Bioresour. Technol.* 97 (2006) 1061–1085.
- [40] V.K. Gupta, R. Jain, S. Varshney, Electrochemical removal of the hazardous dye Reactofix Red 3 BFN from industrial effluents, *J. Colloid Interface Sci.* 312 (2007) 292–296.
- [41] S.H. Lin, C.F. Peng, Continuous treatment of textile wastewater by combined coagulation, electrochemical oxidation and activated sludge, *Water Res.* 30 (1996) 587–592.
- [42] N. Al Bastaki, Removal of methyl orange dye and Na<sub>2</sub>SO<sub>4</sub> salt from synthetic waste water using reverse osmosis, *Chem. Eng. Process.* 43 (2004) 1561–1567.
- [43] M. Marcucci, G. Nosenzo, G. Capannelli, I. Ciabatti, D. Corrieri, G. Ciardelli, Treatment and reuse of textile effluents based on new ultrafiltration and other membrane technologies, *Desalination* 138 (2001) 75–82.
- [44] S. Sostar Turk, M. Simonic, I. Petrinic, Wastewater treatment after reactive printing, *Dyes Pigm.* 64 (2005) 147–152.
- [45] M.K. Purkait, A. Maiti, S. DasGupta, Removal of Congo red using activated carbon and its regeneration, *J. Hazard. Mater.* 145 (2007) 287–295.
- [46] F. Banat, S. Al Asheh, L. Al Makhadmeh, Evaluation of the use of raw and activated date pits as potential adsorbents for dye containing waters, *Process Biochem.* 39 (2003) 193–202.
- [47] S.C.R. Santos, R.A.R. Boaventura, Adsorption modelling of textile dyes by sepiolite, *Appl. Clay Sci.* 42 (2008) 137–145.
- [48] R. Sanghi, B. Bhattacharya, Review on decolorisation of aqueous dye solutions by low cost adsorbents, *Color. Technol.* 118 (2002) 256–269.
- [49] K.R. Ramakrishna, T. Viraraghavan, Dye removal using low cost adsorbents, *Water Sci. Technol.* 36 (1997) 189–196.
- [50] J.W. Hassler, Activated Carbon, Chemical Publishing Company, Inc., New York, 1963.
- [51] R.C. Bansal, J.B. Donet, F. Stoeckli, Active Carbon, Marcel Dekker, New York, 1988.
- [52] P.J.M. Carrott, M.M.L. Ribeiro Carrott, P.A.M. Mourão, R.P. Lima, Preparation of activated carbons from cork by physical activation in carbon dioxide, *Adsorpt. Sci. Technol.* 21 (2003) 669–681.
- [53] M.A. Lillo Rodenas, J.P. Marco Lozar, D. Cazorla Amoros, A. Linares Solano, Activated carbons prepared by pyrolysis of mixtures of carbon precursor/alkaline hydroxide, *J. Anal. Appl. Pyrolysis* 80 (2007) 166–174.
- [54] N.H. Phan, S. Rio, C. Faur, L. Le Coq, P. Le Cloirec, T.H. Nguyen, Production of fibrous activated carbons from natural cellulose (jute, coconut) fibers for water treatment applications, *Carbon* 44 (2006) 2569–2577.
- [55] P.J.M. Carrott, M.M.L. Ribeiro Carrott, R.A. Roberts, Physical adsorption of gases by microporous carbons, *Colloids Surf.* 58 (1991) 385–400.
- [56] I.N. Najm, V.L. Snoeyink, B.W.J. Lykins, J.Q. Adams, Using powdered activated carbon: a critical review, *J. Am. Water Works Assn.* 83 (1991) 65–76.
- [57] Y. Al Degs, M.A.M. Khraisheh, S.J. Allen, M.N. Ahmad, Effect of carbon surface chemistry on the removal of reactive dyes from textile effluent, *Water Res.* 34 (2000) 927–935.
- [58] Y. Al Degs, M.A.M. Khraisheh, S.J. Allen, M.N.A. Ahmad, Sorption behaviour of cationic and anionic dyes from aqueous solution on different types of activated carbons, *Sep. Sci. Technol.* 36 (2001) 91–102.
- [59] C. Pelekani, V.L. Snoeyink, Competitive adsorption between Atrazine and Methylene Blue on activated carbon: the importance of pore size distribution, *Carbon* 38 (2000) 1423–1436.
- [60] G.M. Walker, L.R. Weatherley, Kinetics of acid dye adsorption on GAC, *Water Res.* 33 (1999) 1895–1899.
- [61] G. McKay, Adsorption of dyestuffs from aqueous solutions with activated carbon I: equilibrium and batch contact time studies, *J. Chem. Technol. Biotechnol.* 32 (1982) 759–772.
- [62] E.N. El Qada, S.J. Allen, G.M. Walker, Adsorption of basic dyes from aqueous solution onto activated carbons, *Chem. Eng. J.* 135 (2008) 174–184.
- [63] S. Karaca, A. Gürses, M. Açıkıldız, M. Ejder (Korucu), Adsorption of cationic dye from aqueous solutions by activated carbon, *Microporous Mesoporous Mater.* 115 (2008) 376–382.
- [64] Y.S. Al Degs, M.I. El Barghouthi, A.H. El Sheikh, G.M. Walker, Effect of solution pH, ionic strength, and temperature on adsorption behavior of reactive dyes on activated carbon, *Dyes Pigm.* 77 (2008) 16–23.
- [65] V. Gómez, M.S. Larrechi, M.P. Callao, Kinetic and adsorption study of acid dye removal using activated carbon, *Chemosphere* 69 (2007) 1151–1158.
- [66] H.M. Freeman, Standard Handbook of Hazardous Waste Treatment and Disposal, second ed., McGraw Hill, New York, 1989.
- [67] E.J. Kilduff, C.J. King, Effect of carbon adsorbent surface properties on the uptake and solvent regeneration of phenol, *Ind. Eng. Chem. Res.* 36 (1997) 1603–1613.
- [68] R.J. Martin, W.J. Ng, The repeated exhaustion and regeneration of activated carbon, *Water Res.* 21 (1987) 961–965.
- [69] R.M. Narbaitz, J. Cen, Electrochemical regeneration of granular activated carbon, *Water Res.* 28 (1994) 1771–1778.
- [70] G. Newcombe, M. Drikas, Chemical regeneration of granular activated carbon from an operating water treatment plant, *Water Res.* 27 (1993) 161–165.
- [71] S. Notthakum, J.C. Crittenden, D.W. Hand, D.L. Perram, M.E. Mullins, Regeneration of adsorbents using heterogeneous advanced oxidation, *J. Environ. Eng. ASCE* 119 (1993) 695–714.
- [72] M.A. Rollar, M.T. Suidan, W.H. Goss, S.A. Vargo, Regeneration of five activated carbon with methanol, *J. Environ. Eng. ASCE* 108 (1982) 1361–1377.
- [73] E.A. Taiwo, A. Adesina, Electrochemical regeneration of a native activated carbon, *Chem. Biochem. Eng. Q.* 19 (2005) 269–273.
- [74] M.H. Zhou, L.C. Lei, Electrochemical regeneration of activated carbon loaded with *p* nitrophenol in a fluidized electrochemical reactor, *Electrochim. Acta* 51 (2005) 4489–4496.
- [75] M. Streat, J.W. Patrick, M.J. Pérez, Sorption of phenol and para chlorophenol from water using conventional and novel activated carbons, *Water Res.* 29 (1995) 467–472.
- [76] I. Ali, V.K. Gupta, Advances in water treatment by adsorption technology, *Nat. Protoc.* 1 (2007) 2661–2667.
- [77] B.H. Hameed, A.L. Ahmad, K.N.A. Latiff, Adsorption of basic dye (methylene blue) onto activated carbon prepared from rattan sawdust, *Dyes Pigm.* 75 (2007) 143–149.

- [78] V.K. Garg, M. Amita, R. Kumar, R. Gupta, Basic dye (methylene blue) removal from simulated wastewater by adsorption using Indian Rosewood sawdust: a timber industry waste, *Dyes Pigm.* 63 (2004) 243–250.
- [79] M. Özacar, A.I. Sengil, Adsorption of metal complex dyes from aqueous solutions by pine sawdust, *Bioresour. Technol.* 96 (2005) 791–795.
- [80] G. McKay, J.F. Porter, G.R. Prasad, The removal of dye colours from aqueous solutions by adsorption on low cost materials, *Water Air Soil Pollut.* 114 (1999) 423–438.
- [81] Y.S. Ho, T.H. Chiang, Y.M. Hsueh, Removal of basic dye from aqueous solutions using tree fern as a biosorbent, *Process Biochem.* 40 (2005) 119–124.
- [82] M. Nassar, Y.H. Magdy, Removal of different basic dyes from aqueous solutions by adsorption on palm fruit bunch particles, *Chem. Eng. J.* 66 (1997) 223–226.
- [83] G. Sun, X. Xu, Sunflower stalk as adsorbents for color removal from textile wastewater, *Ind. Eng. Chem. Res.* 36 (1997) 808–812.
- [84] B.H. Hameed, Spent tea leaves: a new non conventional and low cost adsorbent for removal of basic dye from aqueous solutions, *J. Hazard. Mater.* 161 (2009) 753–759.
- [85] B.H. Hameed, Removal of cationic dye from aqueous solution using jackfruit peel as non conventional low cost adsorbent, *J. Hazard. Mater.* 162 (2009) 344–350.
- [86] S.J. Allen, Q. Gan, R. Matthews, P.A. Johnson, Comparison of optimised isotherm models for basic dye adsorption by kudzu, *Bioresour. Technol.* 88 (2003) 143–152.
- [87] C. Namasivayam, M. Dinesh Kumar, K. Selvi, R. Ashruffunissa Begum, T. Vanathi, R.T. Yamuna, 'Waste' coir pith a potential biomass for the treatment of dyeing wastewaters, *Biomass Bioenergy* 21 (2001) 477–483.
- [88] M. Arami, N.Y. Limaee, N.M. Mahmoodi, N.S. Tabrizi, Equilibrium and kinetics studies for the adsorption of direct and acid dyes from aqueous solution by soy meal hull, *J. Hazard. Mater.* 135 (2006) 171–179.
- [89] K.S. Chou, J.C. Tsai, C.T. Lo, The adsorption of Congo red and vacuum pump oil by rice hull ash, *Bioresour. Technol.* 78 (2001) 217–219.
- [90] Y.S. Ho, G. McKay, Sorption of dyes and copper ions onto biosorbents, *Process Biochem.* 38 (2003) 1047–1061.
- [91] B. Chen, C.W. Hui, G. McKay, Film pore diffusion modeling and contact time optimisation for the adsorption of dyestuffs on pith, *Chem. Eng. J.* 84 (2001) 77–94.
- [92] O. Hamdaoui, Batch study of liquid phase adsorption of methylene blue using cedar sawdust and crushed brick, *J. Hazard. Mater.* 135 (2006) 264–273.
- [93] C. Namasivayam, R. Radhika, S. Suba, Uptake of dyes by a promising locally available agricultural solid waste: coir pith, *Waste Manage.* 21 (2001) 381–387.
- [94] L.C. Morais, O.M. Freitas, E.P. Gonçalves, L.T. Vasconcelos, C.G. Gonzalez Beça, Reactive dyes removal from wastewaters by adsorption on eucalyptus bark: variables that define the process, *Water Res.* 33 (1999) 979–988.
- [95] F. Ferrero, Dye removal by low cost adsorbents: hazelnut shells in comparison with wood sawdust, *J. Hazard. Mater.* 142 (2007) 144–152.
- [96] V.K. Garg, R. Gupta, A.B. Yadav, R. Kumar, Dye removal from aqueous solution by adsorption on treated sawdust, *Bioresour. Technol.* 89 (2003) 121–124.
- [97] Y.S. Ho, G. McKay, A kinetic study of dye sorption by biosorbent waste product pith, *Resour. Conserv. Recycl.* 25 (1999) 171–193.
- [98] M. Hasnain Isa, L. Siew Lang, F.A.H. Asaari, H.A. Aziz, N. Azam Ramli, J.P.A. Dhas, Low cost removal of disperse dyes from aqueous solution using palm ash, *Dyes Pigm.* 74 (2007) 446–453.
- [99] M.S. El Geundi, I.H. Aly, Equilibrium studies during the adsorption of acid dyestuffs onto maize cob, *Adsorpt. Sci. Technol.* 9 (1992) 121–129.
- [100] G. Annadurai, R.S. Juang, D.J. Lee, Use of cellulose based wastes for adsorption of dyes from aqueous solutions, *J. Hazard. Mater.* B92 (2002) 263–274.
- [101] Rajeshwarisivaraj, C. Namasivayam, K. Kadirvelu, Orange peel as an adsorbent in the removal of acid violet 17 (acid dye) from aqueous solutions, *Waste Manage.* 21 (2001) 105–110.
- [102] C. Namasivayam, D. Prabha, M. Kumutha, Removal of direct red and acid brilliant blue by adsorption on banana pith, *Bioresour. Technol.* 64 (1998) 77–79.
- [103] S.D. Khattri, M.K. Singh, Colour removal from dye wastewater using sugar cane dust as an adsorbent, *Adsorpt. Sci. Technol.* 17 (1999) 269–282.
- [104] S.D. Khattri, M.K. Singh, Colour removal from synthetic dye waste water using a bioadsorbent, *Water Air Soil Pollut.* 120 (2000) 283–294.
- [105] V.K. Garg, R. Kumar, R. Gupta, Removal of malachite green dye from aqueous solution by adsorption using agro industry waste: a case study of *Prosopis cineraria*, *Dyes Pigm.* 62 (2004) 1–10.
- [106] F.A. Batzias, D.K. Sidiras, Dye adsorption by calcium chloride treated beech sawdust in batch and fixed bed systems, *J. Hazard. Mater.* B114 (2004) 167–174.
- [107] S.E. Bailey, T.J. Olin, M. Bricka, D.D. Adrian, A review of potentially low cost sorbents for heavy metals, *Water Res.* 33 (1999) 2469–2479.
- [108] T. Robinson, B. Chandran, P. Nigam, Removal of dyes from an artificial textile dye effluent by two agricultural waste residues, corn cob and barley husk, *Environ. Int.* 28 (2002) 29–33.
- [109] P. Nigam, G. Armour, I.M. Banat, D. Singh, R. Marchant, Physical removal of textile dyes from eluents and solid state fermentation of dye adsorbed agricultural residues, *Bioresour. Technol.* 72 (2000) 219–226.
- [110] R.M. Gong, S.X. Zhua, D.M. Zhang, J. Chen, S.J. Ni, R. Guan, Adsorption behavior of cationic dyes on citric acid esterifying wheat straw: kinetic and thermodynamic profile, *Desalination* 230 (2008) 220–228.
- [111] R.M. Gong, Y.B. Jin, J. Sun, K.D. Zhong, Preparation and utilization of rice straw bearing carboxyl groups for removal of basic dyes from aqueous solution, *Dyes Pigm.* 76 (2008) 519–524.
- [112] R.M. Gong, Y.B. Jin, J. Chen, Y. Hu, J. Sun, Removal of basic dyes from aqueous solution by sorption on phosphoric acid modified rice straw, *Dyes Pigm.* 73 (2007) 332–337.
- [113] B.C. Oei, S. Ibrahim, S.B. Wang, H.M. Ang, Surfactant modified barley straw for removal of acid and reactive dyes from aqueous solution, *Bioresour. Technol.* 100 (2009) 4292–4295.
- [114] W.T. Tsai, C.Y. Chang, S.L. Lee, Preparation and characterization of activated carbons from corn cob, *Carbon* 35 (1997) 1198–1200.
- [115] D. Savova, E. Apak, E. Ekinci, F. Yardim, N. Petrova, T. Budinova, M. Razvigorova, V. Minkova, Biomass conversion to carbon adsorbents and gas, *Biomass Bioenergy* 21 (2001) 133–142.
- [116] M. Lanzetta, C. Di Blasi, Pyrolysis kinetics of wheat and corn straw, *J. Anal. Appl. Pyrolysis* 44 (1998) 181–192.
- [117] M. Ahmedna, W.E. Marshall, R.M. Rao, Production of granular activated carbons from select agricultural byproducts and evaluation of their physical, chemical and adsorption properties, *Bioresour. Technol.* 71 (2000) 113–123.



- [118] G.H. Oh, C.R. Park, Preparation and characteristics of rice straw based porous carbons with high adsorption capacity, *Fuel* 81 (2002) 327–336.
- [119] B.G. Prakash Kumar, Lima Rose Miranda, M. Velan, Adsorption of Bismark Brown dye on activated carbons prepared from rubberwood sawdust (*Hevea brasiliensis*) using different activation methods, *J. Hazard. Mater.* B126 (2005) 63–70.
- [120] P.K. Malik, Dye removal from wastewater using activated carbon developed from sawdust: adsorption equilibrium and kinetics, *J. Hazard. Mater.* B113 (2004) 81–88.
- [121] P.K. Malik, Use of activated carbons prepared from sawdust and rice husk for adsorption of acid dyes: a case study of Acid Yellow 36, *Dyes Pigm.* 56 (2003) 239–249.
- [122] R.S. Juang, F.C. Wu, R.L. Tseng, Characterization and use of activated carbons prepared from bagasses for liquid phase adsorption, *Colloids Surf. A* 201 (2002) 191–199.
- [123] M. Valix, W.H. Cheung, G. McKay, Preparation of activated carbon using low temperature carbonisation and physical activation of high ash raw bagasse for acid dye adsorption, *Chemosphere* 56 (2004) 493–501.
- [124] A.E. Putun, N. Ozbay, E.P. Onal, E. Putun, Fixed bed pyrolysis of cotton stalk for liquid and solid products, *Fuel Process. Technol.* 86 (2005) 1207–1219.
- [125] I.A.W. Tan, A.L. Ahmad, B.H. Hameed, Adsorption of basic dye on high surface area activated carbon prepared from coconut husk: equilibrium, kinetic and thermodynamic studies, *J. Hazard. Mater.* 154 (2008) 337–346.
- [126] Y.P. Guo, S.F. Yang, W.Y. Fu, J.R. Qi, R.Z. Li, Z.C. Wang, H.D. Xu, Adsorption of malachite green on micro- and mesoporous rice husk based active carbon, *Dyes Pigm.* 56 (2003) 219–229.
- [127] M.M. Mohamed, Acid dye removal: comparison of surfactant modified mesoporous FSM 16 with activated carbon derived from rice husk, *J. Colloid Interface Sci.* 272 (2004) 28–34.
- [128] W. Li, J.H. Peng, L.B. Zhang, H.Y. Xia, N. Li, K.B. Yang, X.Y. Zhu, Investigations on carbonization processes of plain tobacco stems and H<sub>3</sub>PO<sub>4</sub> impregnated tobacco stems used for the preparation of activated carbons with H<sub>3</sub>PO<sub>4</sub> activation, *Ind. Crops Prod.* 28 (2008) 73–80.
- [129] T. Yang, A.C. Lua, Characteristics of activated carbons prepared from pistachio nut shells by physical activation, *J. Colloid Interface Sci.* 267 (2003) 408–417.
- [130] M. Ahmedna, W.E. Marshall, A.A. Hussein, R.M. Rao, I. Goktepe, The use of nutshell carbons in drinking water filters for removal of trace metals, *Water Res.* 38 (2004) 1062–1068.
- [131] T. Tay, S. Ucar, S. Karagöz, Preparation and characterization of activated carbon from waste biomass, *J. Hazard. Mater.* 165 (2009) 481–485.
- [132] I.A.W. Tan, A.L. Ahmad, B.H. Hameed, Enhancement of basic dye adsorption uptake from aqueous solutions using chemically modified oil palm shell activated carbon, *Colloids Surf. A* 318 (2008) 88–96.
- [133] I.A.W. Tan, B.H. Hameed, A.L. Ahmad, Equilibrium and kinetic studies on basic dye adsorption by oil palm fibre activated carbon, *Chem. Eng. J.* 127 (2007) 111–119.
- [134] T. Zhang, W.P. Walawender, L.T. Fan, M. Fan, D. Daugaard, R.C. Brown, Preparation of activated carbon from forest and agricultural residues through CO<sub>2</sub> activation, *Chem. Eng. J.* 105 (2004) 53–59.
- [135] O. Ioannidou, A. Zabaniotou, Agricultural residues as precursors for activated carbon production – a review, *Renewable Sustainable Energy Rev.* 11 (2007) 1966–2005.
- [136] W.T. Tsai, C.Y. Chang, S.L. Lee, A low cost adsorbent from agricultural waste corn cob by zinc chloride activation, *Bioresour. Technol.* 64 (1998) 211–217.
- [137] M. Fan, W. Marshall, D. Daugaard, R.C. Brown, Steam activation of chars produced from oat hulls and corn stover, *Bioresour. Technol.* 93 (2004) 103–107.
- [138] V. Minkova, M. Razvigorova, E. Bjornbom, R. Zanzi, T. Budinova, N. Petrov, Effect of water vapour and biomass nature on the yield and quality of the pyrolysis products from biomass, *Fuel Process. Technol.* 70 (2001) 53–61.
- [139] V. Minkova, S.P. Marinov, R. Zanzi, E. Bjornbom, T. Budinova, M. Stefanova, L. Lakov, Thermochemical treatment of biomass in a flow of steam or in a mixture of steam and carbon dioxide, *Fuel Process. Technol.* 62 (2000) 45–52.
- [140] B.S. Girgis, S.S. Yunis, A.M. Soliman, Characteristics of activated carbon from peanut hulls in relation to conditions of preparation, *Mater. Lett.* 57 (2002) 164–172.
- [141] A. Abdel Nasser, El Hendawy, S.E. Samra, B.S. Girgis, Adsorption characteristics of activated carbons obtained from corncobs, *Colloids Surf. A* 180 (2001) 209–221.
- [142] J.J.M. Órfão, A.I.M. Silva, J.C.V. Pereira, S.A. Barata, I.M. Fonseca, P.C.C. Faria, M.F.R. Pereira, Adsorption of a reactive dye on chemically modified activated carbons – Influence of pH, *J. Colloid Interface Sci.* 296 (2006) 480–489.
- [143] P.C.C. Faria, J.J.M. Órfão, M.F.R. Pereira, Adsorption of anionic and cationic dyes on activated carbons with different surface chemistries, *Water Res.* 38 (2004) 2043–2052.
- [144] A.A. Attia, W.E. Rashwan, S.A. Khedr, Capacity of activated carbon in the removal of acid dyes subsequent to its thermal treatment, *Dyes Pigm.* 69 (2006) 128–136.
- [145] C. Pelekani, V.L. Snoeyink, A kinetic and equilibrium study of competitive adsorption between atrazine and Congo red dye on activated carbon: the importance of pore size distribution, *Carbon* 39 (2001) 25–37.
- [146] L.S. Chan, W.H. Cheung, G. McKay, Adsorption of acid dyes by bamboo derived activated carbon, *Desalination* 218 (2008) 304–312.
- [147] R.S. Juang, R.L. Tseng, F.C. Wu, Role of microporosity of activated carbons on their adsorption abilities for phenols and dyes, *Adsorption* 7 (2001) 65–72.
- [148] N. Kannan, M.M. Sundaram, Kinetics and mechanism of removal of methylene blue by adsorption on various carbons a comparative study, *Dyes Pigm.* 51 (2001) 25–40.
- [149] C. Akmal Basar, Y. Onal, T. Kilicer, D. Eren, Adsorptions of high concentration malachite green by two activated carbons having different porous structures, *J. Hazard. Mater.* 127 (2005) 73–80.
- [150] A. Jumasiah, T.G. Chuah, J. Gimbon, T.S.Y. Choong, I. Azni, Adsorption of basic dye onto palm kernel shell activated carbon: sorption equilibrium and kinetics studies, *Desalination* 186 (2005) 57–64.
- [151] I.A.W. Tan, A.L. Ahmad, B.H. Hameed, Adsorption of basic dye using activated carbon prepared from oil palm shell: batch and fixed bed studies, *Desalination* 225 (2008) 13–28.
- [152] B.H. Hameed, F.B.M. Daud, Adsorption studies of basic dye on activated carbon derived from agricultural waste: *Hevea brasiliensis* seed coat, *Chem. Eng. J.* 139 (2008) 48–55.
- [153] R. Malik, D.S. Ramteke, S.R. Wate, Adsorption of malachite green on groundnut shell waste based powdered activated carbon, *Waste Manage.* 27 (2007) 1129–1138.

- [154] N. Thinakaran, P. Panneerselvam, P. Baskaralingam, D. Elango, S. Sivanesan, Equilibrium and kinetic studies on the removal of Acid Red 114 from aqueous solutions using activated carbons prepared from seed shells, *J. Hazard. Mater.* 158 (2008) 142–150.
- [155] S. Senthilkumaar, P. Kalaamani, K. Porkodi, P.R. Varadarajan, C.V. Subburaam, Adsorption of dissolved Reactive red dye from aqueous phase onto activated carbon prepared from agricultural waste, *Bioresour. Technol.* 97 (2006) 1618–1625.
- [156] B. Karagozoglu, M. Tasdemir, E. Demirbas, M. Koby, The adsorption of basic dye (Astrazon Blue FGRL) from aqueous solutions onto sepiolite, fly ash and apricot shell activated carbon: kinetic and equilibrium studies, *J. Hazard. Mater.* 147 (2007) 297–306.
- [157] C.A. Başar, Applicability of the various adsorption models of three dyes adsorption onto activated carbon prepared waste apricot, *J. Hazard. Mater.* B135 (2006) 232–241.
- [158] A. Khaled, A. El Nemr, A. El Sikaily, O. Abdelwahab, Removal of Direct N Blue 106 from artificial textile dye effluent using activated carbon from orange peel: Adsorption isotherm and kinetic studies, *J. Hazard. Mater.* 165 (2009) 100–110.
- [159] A. Gürses, Ç. Doğar, S. Karaca, M. Açıkyıldız, R. Bayrak, Production of granular activated carbon from waste *Rosa canina* sp. seeds and its adsorption characteristics for dye, *J. Hazard. Mater.* B131 (2006) 254–259.
- [160] C. Namasivayam, D. Kavitha, Removal of Congo red from water by adsorption onto activated carbon prepared from coir pith, an agricultural solid waste, *Dyes Pigm.* 54 (2002) 47–58.
- [161] W.T. Tsai, C.Y. Chang, M.C. Lin, S.F. Chien, H.F. Sun, M. F. Hsieh, Adsorption of acid dye onto activated carbon prepared from agricultural waste bagasse by  $\text{ZnCl}_2$  activation, *Chemosphere* 45 (2001) 51–58.

# Liquefaction and Gasification of Cereal Straws

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Liquefaction is an effective method to convert straws into liquid products, which are potential intermediates for the production of fuels and chemicals. Based on the purpose and the process, liquefaction can be classified into hydrothermal liquefaction and solvolytic liquefaction. Hydrothermal liquefaction, which is a traditional process, mainly operates under high temperature and high pressure to obtain bio-oil. Recently, the solvolytic liquefaction, which operates under moderate and low temperatures or even under atmospheric pressure, has gained more and more attention. Products from solvolytic liquefaction can be applied as fuels, fuel additives, or preparation of polymer material. Gasification of feedstock is a process carried out at high temperature in certain medium to produce gas. Gasification could be classified into two types according to the reaction medium: thermal gasification and hydrothermal gasification. The conventional gasification process, thermal gasification, occurs in gas agents to produce syngas. Hydrothermal gasification is a novel gasification process that occurs in subcritical and supercritical water, and  $H_2$  is the principal product gas.

## 7.4.1 HYDROTHERMAL LIQUEFACTION

Hydrothermal liquefaction is to control the reaction rate and reaction mechanisms to produce bio-oil by using pressure, gases, and/or catalysts. Hydrothermal liquefaction processes normally operate at high temperature (200–450 °C) and high pressure (>1 MPa) in hot compressed water or organic solvents such as alcohols (mainly methanol, ethanol, propanol, and butanol) and acetone, and typical yields of liquid products are between 20 and 60%. More recently, supercritical fluids have gained increasing attention in hydrothermal liquefaction. The advantage of hydrothermal liquefaction is that the bio-oil produced by liquefaction is not miscible with water and has a lower oxygen content,

which has a higher energy content than the pyrolysis-derived oils [1].

Many complicated chemical reactions are involved in hydrothermal liquefaction, which corresponded to the following steps [2]:

1. solvolysis of the feedstock;
2. depolymerization of the main components cellulose, hemicelluloses, and lignin;
3. chemical and thermal decomposition of monomers and smaller molecules by bond ruptures, dehydration, and decarboxylation;
4. degradation of oxygen-containing functional groups, such as hydroxyl groups and carboxyl groups, in the presence of hydrogen ( $H_2$ ).

More details about the liquefaction reaction can be found in related reviews [2–4]. Depolymerization of cellulose and hemicelluloses is through rupture of ring bonds or rearrangement of the formed monosaccharide [5]. Depolymerization of cellulose in acid aqueous media occurs at above 240 °C, whereas depolymerization of hemicelluloses occurs at 120–180 °C. Degradation products have a multitude of oxygen-containing functional groups, and only a few of them are able to reduce oxygen by cleavage of carbon dioxide ( $CO_2$ ) further. Therefore, in the presence of  $H_2$ , it is essential to avoid repolymerization in degradation of functional groups.

Depolymerization of lignin produces numerous different substituted phenols. Free phenoxyl radicals are formed by thermal decomposition of lignin at above 252 °C, which have a random tendency to form solid residue through condensation or repolymerization. Therefore, prolonging holding time involves increase of the repolymerization, which leads to the decrease of the yield of liquid products [4]. Recently, the reaction pathways of lignin [6] and cellulose [7] in supercritical water/phenol solutions under high temperature were published by Fang et al. (Figs. 7.4.1

and 7.4.2). Lignin was decomposed mainly by homogenous hydrolysis and pyrolysis reactions, and the homogenous reactions inhibited repolymerization of the phenolics compounds and promoted the formation of oil. The reaction paths for lignin included four different phases: oil phase, aqueous phase, gas phase, and solid residue phase. During heating of cellulose, homogeneous hydrolysis and decomposition reactions occurred almost simultaneously, and the reaction pathways and products varied with different heating rates and catalyst. For the addition of catalysts  $\text{Na}_2\text{CO}_3$  or Ni, the main products were oil or gas.

Liquefaction products (bio-oil) from liquefaction contain a complex mixture of volatile organic acids, alcohols, aldehydes, ethers, esters, ketones, furans, phenols, hydrocarbons, and nonvolatile components [8], and the specific composition of liquefaction products was dependent on feedstock. In liquefaction of low-input high-diversity grassland perennials (LIHD), the liquid products were mainly composed of aromatic hydrocarbons, ketones, aldehydes, carboxylic acids, esters, nitrogenated compounds, and their derivatives; liquid products from lignin of LIHD had a high concentration of monoaromatic compounds, whereas liquid products from pretreated LIHD contained a significant amount of furfural [9]. Liquid products from corn stover contained mainly 4-ethyl-phenol, 1-(4-hydroxy-3,5-dimethoxyphenyl)-ethanone, desaspidinol, 2-hydroxy-3-methyl-2-cyclopenten-1-one, 2,5-hexanedione, and 1-hydroxy-2-propanone [10]. In subcritical and supercritical 1,4-dioxane-water mixture liquefaction rice straw, the liquefied products were mainly composed of phenolic compounds, ester derivatives, hydrocarbon, organic acids, and alcohols [11]. Gas from liquefaction of corn stover mainly consisted of  $\text{H}_2$ , carbon monoxide ( $\text{CO}$ ), methane ( $\text{CH}_4$ ), and  $\text{CO}_2$ , the content of which were 2.6 4.8, 8 27, 1.1 2.8, and 68 85% of the total gaseous products, respectively [10].

Generally, higher lignin content results in higher residue and lower liquid yield, which was confirmed by Zhang's research [9]. The yield of solid for LIHD lignin at 35.1% was higher than that of LIHD at 1.8%, whereas the yield of liquid for LIHD lignin at 59.7% was lower than that of LIHD at 77.4% [9].

Many researches have been conducted on the influence of the solvent on the liquefaction products. The medium for conventional high-pressure direct liquefaction processes normally were hot-compressed water and organic solvents, which include anthracene oil, alcohols (methanol, ethanol, propanol, and butanol), and acetone [12]. More recently, supercritical fluids including supercritical water, alcohols, and acetone have gained much attention.

In liquefaction of *Verbascum* stalk with supercritical methanol, ethanol, and acetone at 280 °C, the oil yields of 44.4, 43.3, and 60.5% were obtained, respectively [13]. In liquefaction of *Prangmites australis* using organic solvents

(methanol and ethanol) with catalyst (10% NaOH) and without catalyst in an autoclave at 270 °C, the yields from supercritical methanol and ethanol were 38.7 and 53.6%, respectively, compared with 44.2 and 57.6% in the presence of catalyst, respectively [14].

Generally, ethanol appears to be a more effective solvent compared with other n-alcohols (such as methanol, propanol, butanol, and pentanol), which were confirmed by liquefaction of sugarcane bagasse [15, 16] and depolymerization of lignin [17]. However, recent comparative research on the sub- and supercritical liquefaction of rice straw between ethanol/water medium and 2-propanol/water medium in the autoclave showed the maximum yield of bio-oil was obtained at 39.7% when 2-propanol: water volume ratio of 5:5 at 300 °C. In addition, the rice straw liquefied with 2-propanol/water had a slight superiority on higher heating value (HHV) bio-oil than that liquefied with ethanol/water [18]. Recently, critical liquefaction of rice straw in subcritical and supercritical 1,4-dioxane-water mixture was investigated, and the yields of oil were in the range of 29.64–57.30% [11].

Alkaline solutions, such as  $\text{Na}_2\text{CO}_3$ , NaOH,  $\text{K}_2\text{CO}_3$ , KOH, LiOH, RbOH, CsOH, and  $\text{Ca}(\text{OH})_2$ , have been widely used as catalysts in direct liquefaction processes to suppress the formation of char and to enhance the yield of liquid products. Some alkaline catalysts (Rb and Cs) were found effective in altering the chemical composition of the resulted liquid products [12]. In liquefaction of straw, NaOH and  $\text{Na}_2\text{CO}_3$  are used broadly.

By the addition of 10.0% NaOH catalyst in supercritical methanol and ethanol liquefaction, the conversion yield of both *Verbascum* and sunflower increased in different temperatures [19]. Sunflower stalk mill was converted to liquid products by using supercritical organic solvents (methanol, ethanol, and acetone) with NaOH and without catalyst at 290 °C. The conversion yields in supercritical methanol, ethanol, and acetone liquefaction were 40.00, 45.60, and 47.80%, respectively, whereas those in the methanol and ethanol liquefaction with catalyst were 58.03 and 54.17%, respectively [20].

At relatively higher temperatures (310 °C) with the addition of 1.0% of  $\text{Na}_2\text{CO}_3$ , the yield of bio-oil was improved from 33.4 to 47.2% and the quality of the liquid product was enhanced [21]. When catalyst  $\text{Na}_2\text{CO}_3$  was added as catalyst in liquefaction, the catalyst had a positive effect on yields of liquid obtained from the tobacco stalk and especially waste tobacco leaves, but the effect was not significant to the corn stover [22].

During liquefaction of LIHD, catalyst, such as NaOH,  $\text{SO}_4^{2-}/\text{ZrO}_2$ ,  $\text{Al}_2\text{O}_3$ , and  $\text{CaO}/\text{ZrO}_2$ , decreased the yield of the residue from 17–18 to 13–16%, but the effect to the liquid yields were not significant [9].

The higher the temperature, the easier the degradation of the polymers into liquid oil, but further increase of the



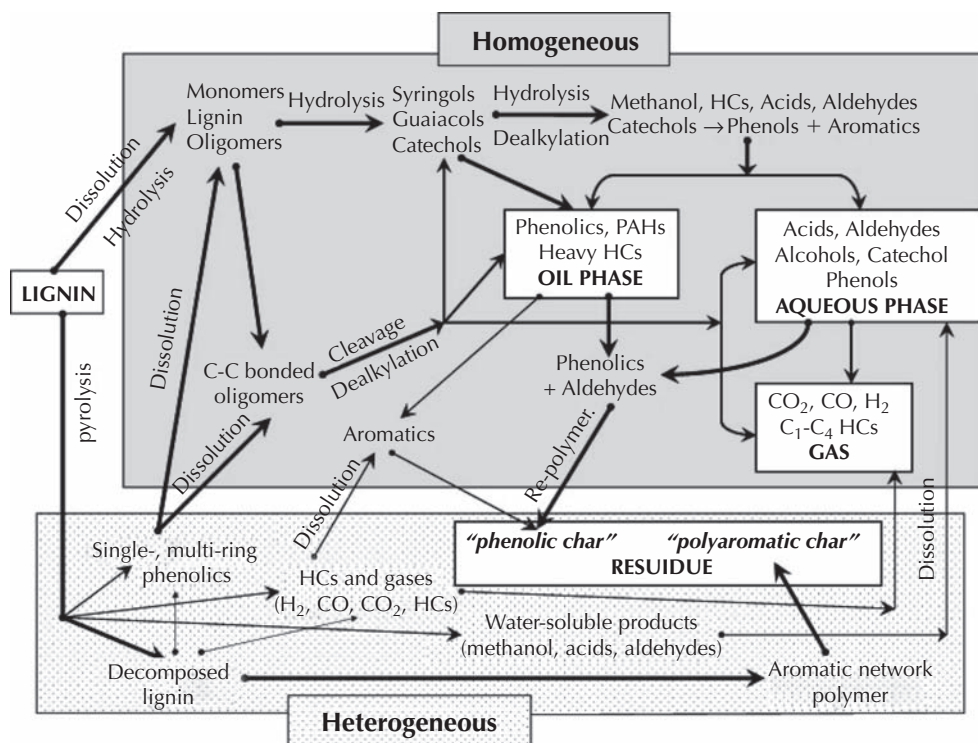


FIGURE 7.4.1 Reaction pathways of lignin hydrothermal liquefaction in supercritical water [6].

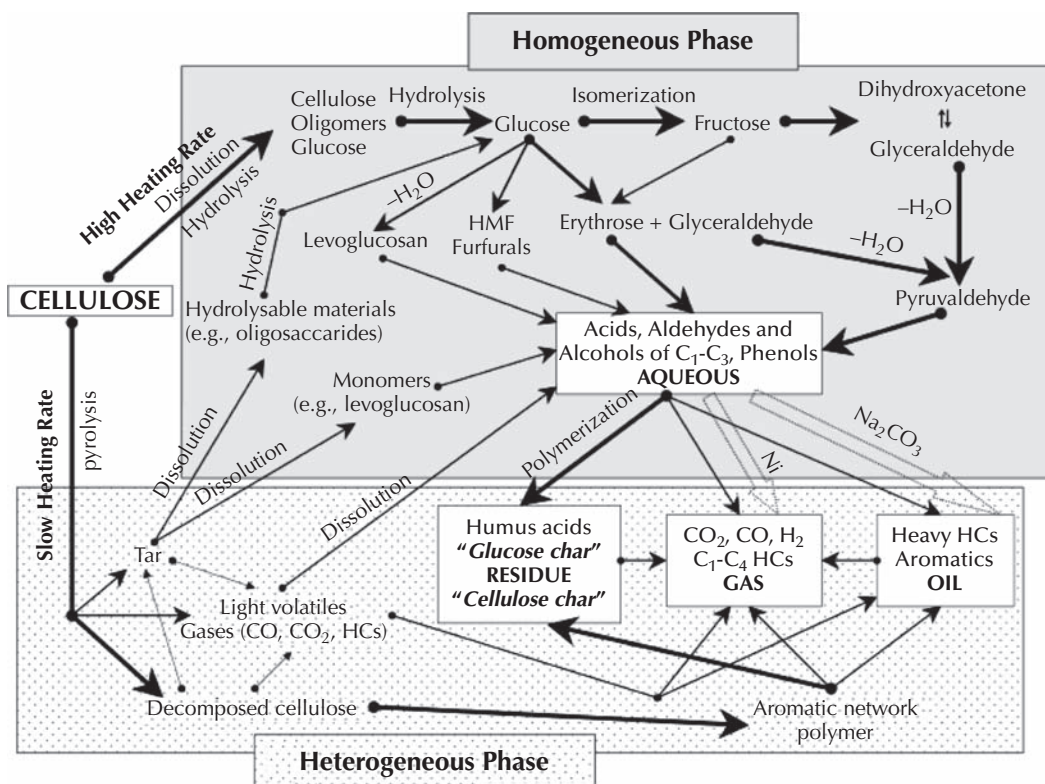


FIGURE 7.4.2 Reaction pathways of cellulose hydrothermal liquefaction in aqueous media [7].



temperature results in the decomposition of lower molecular matter into gaseous products. In liquefaction of high-diversity grassland perennials, the final temperature had an obvious influence on the liquefaction yields. When the final temperature was above the supercritical point of water, with an increase in the temperature, the yield of the liquid fraction decreased obviously, and more gaseous products were produced [9].

In liquefaction of rice straw in subcritical and supercritical 1,4-dioxane-water, the yield of oil decreased from 49.00 to 33.01% with the temperature increase from 260 to 320 °C, whereas the yield of residue decreased from 12.18 to 7.56% with the temperature increase from 260 to 340 °C. The reason can be explained by hydrolysis and repolymerization reactions. During the liquefaction, feedstock decomposed and depolymerized to fragments of low molecular weight, and then these unstable fragments produced new polymer through condensation, cyclization, and polymerization, which is enhanced at high temperature [11].

In liquefaction of corncobs in subcritical and supercritical water for 10 min, the liquefaction rate increased from 68 to 82% when the temperature was increased from 300°C to 375 °C [23]. Similarly, the yields of supercritical methanol liquefaction of sunflower stalk increased from 38.52 to 58.03% as the temperature increased from 250 to 290 °C in the presence of catalyst [20].

In subcritical and supercritical liquefaction of rice straw with mixture (ethanol-water and 2-propanol-water), the yield of residue decreased with an increase in the reaction temperature when the temperature was below 300 °C, and it increased with an increase in the temperature when the temperature was above 300 °C, whereas the HHV of bio-oil increased with an increase in the temperature from 260 to 350 °C [18].

In liquefaction of cellulose with  $\text{Na}_2\text{CO}_3$ , a high heating rate leads to the presence of homogeneous phase, which increases the oil yields [7]. During liquefaction of corn stover, composition of the liquid products was not influenced by the heating rate. However, the yield of liquid products increased with an increase in the heating rate. The liquid yield increased significantly from 51.53 to 69.71% when the heating rate was increased from 5 to 140 °C/min, and the yield of solid residue also decreased from 18.20 to ~9% of the total mass, and gas products were decreased [10].

During liquefaction of LIHD, the highest liquid yield was achieved within 6 and 1 min in the residence time at 300 and 374 °C, respectively, and prolonging residence time resulted in lower liquid yield [9]. In liquefaction of rice straw in subcritical and supercritical 1,4-dioxane-water, the yield of oil decreased from 57.30 to 44.00% when the resistance time was prolonged from 0 to 20 min, owing to the formation of residue by repolymerization, condensation, cyclization, and polymerization [11].

The liquid product of hydrothermal liquefaction could be separated into a light and a heavy fraction, which can be used in different energetic applications. One possible application is power generation, such as cocombustion in a coal-fired power, another option is oil production.

Bio-oil from liquefaction has the disadvantages of low heating value, incompatibility with conventional fuels, high solid contents, high viscosity, incomplete volatility, and chemical instability. Therefore, it must be upgraded when used as diesel and gasoline fuels [24]. Generally, there are three different upgrading routes: hydrodeoxygenation, zeolite upgrading, and forming emulsions with the diesel fuel [1]. Bio-oil produced from hydrotreating is stable, has high energy, and is noncorrosive. In zeolite upgrading, extensive coking is produced on the catalyst surface. Bio-oil can form emulsions with diesel fuel, but this fuel is highly corrosive. As for steam reforming, the thermal efficiency is low for syngas production and the subsequent conversion into fuels.

A biocrude was produced by hydrothermal upgrading process with feedstock containing straws, which consisted of 45% liquid products (wt% of input material, dry, and without ash), 25% gas (>90%  $\text{CO}_2$ ), 20%  $\text{H}_2\text{O}$ , and 10% solved organic materials. The heating value was 30–35 MJ/kg, the H/C ratio was 1:1, the oxygen content was between 10 and 18%, and the thermal efficiency for one variant of this process amounted to 74.9% [25].

## 7.4.2 SOLVOLYTIC LIQUEFACTION

In solvolytic liquefaction process, feedstocks are liquefied in organic solvent (commonly polyhydric alcohols) in the presence of acid catalyst under mild temperature (<200 °C) and pressures. The liquid product-introduced hydroxyl groups are potential chemical raw materials, and some polyester and polyurethane are produced from the liquefied polyol product furthermore [26].

In solvolytic liquefaction, degradation and repolymerization are the principal reactions occurring in the process. Degradation makes the straw to decompose and reduces the residue percentage, whereas repolymerization of the degraded products produces insoluble material and increases the residue percentage. At the earlier stage, degradation gets the superiority, resulting in the residue decreasing rapidly. However, at the later stage, repolymerization is enhanced gradually with an increase of small molecules in the reaction system; small molecules are repolymerized into the insoluble polymer. Therefore, the residue content descends slowly at the later stage of the liquefaction, and the residue content could increase under certain conditions.

The chemical changes in solvolytic liquefaction are rather complicated. To elucidate the reaction, liquefaction of related model material of feedstock, including cellulose (glucose), hemicelluloses (xylose), and lignins, have been

studied. The model material liquefied in polyhydric alcohols showed the following:

1. Glucose reacted with glycol to produce glycol glucoside in the presence of concentrated sulfuric acid, glycol glucoside degraded into 5-HMF as intermediates, and the intermediates could be further decomposed to produce levulinic acid and its derivative; on the other hand, the intermediates produced polymer, which repolymerized into insoluble residue [27].
2. Xylose combined with glycol and then degraded into xyloside and glycol xyloside, and xyloside degraded into furfural furthermore. Glycol xyloside self-polymerized or polymerized with glycol, producing the insoluble residue [28].
3. Alkali lignin could be easily liquefied, and the final products contained high levels of fatty acid methyl ester and aromatic ester, some benzene derivative, and less glycol byproduct [28].

Bagasse was liquefied in ethylene glycol (EG), and the crude product was separated into three fractions and characterized by infrared, gel permeation chromatography, elemental analysis, gas chromatography-mass spectrometry, and high-performance liquid chromatography [29]. The fractions contained water-soluble fraction, acetone-soluble fraction, and residue; the residue mainly contained undissolved cellulose and lignin derivatives, the acetone-soluble fraction mainly contained lignin degradation products with high molecular weights, and the water-soluble fraction mainly contained EG, diethylene glycol, EG derivatives, saccharides, alcohols, aldehydes, ketones, phenols, especially some organic acids, and their esters.

Solvolytic liquefaction could be conducted in polyhydric alcohols, such as EG, and cyclic carbonates, such as ethylene carbonate (EC). Compared with the thermochemical liquefaction, the degraded lignin in solvolytic liquefaction is easier to dissolve and not prone to recondensation. Yamada proposed that the liquefaction efficiency was relevant to the dielectric constant values of the solution; higher liquefaction yield was obtained when solvent with higher dielectric constant was used [30].

Polyhydric alcohols, such as polyethylene glycol (PEG 400) and glycerin, were utilized as solvent in liquefaction of straws including bagasse, cotton stalks, and wheat straw. In liquefaction of bagasse and cotton stalks, the residue yield was above 90% even at longer liquefaction time. Therefore, PEG 400 alone was not recommended as the right solvent. The addition of glycerin could improve the liquefaction efficiency, replacing 10% from PEG 400 with glycerin decreased the residue content largely from 18.4 to 6.9% and from 21.3 to 12.1% for bagasse and cotton stalks, respectively [31]. For liquefaction of wheat straw in the mixture of PEG 400 and glycerin, the lowest residue rate was achieved when the solvent contained 80% of PEG 400 and 20% of glycerin [32].

To enhance the liquefaction speed, cyclic carbonates with higher dielectric constant than polyhydric alcohol, such as EC and propylene carbonate (PC), were adopted as the liquefaction reagents. The rate of the liquefaction of cellulose and hardwood in EC or PC was approximately 10 times faster than that in polyhydric alcohol [30]. In the comparison experiment of corn stover, the liquefaction yield reached 80, 74, and 60% in 60 min of reaction when EC, the mixture of PG and glycerol, and EG were used as solvents, respectively [26]; the feedstock liquefied rapidly in EC and approached the maximum liquefaction yield in 0.5 h; whereas the rate of liquefaction in EG was slow, and 15% of the corn stover still remained unliquefied after 2.5 h. This is because EG could react with feedstock to produce CO<sub>2</sub>, which disturbs the process. The mixed solvents, 90% of EG and 10% of EC, were used to achieve a reasonable liquefaction yield in an acceptable way [33].

Decomposition and solvolysis processes are the principal reactions occurring in solvolytic liquefaction; the addition of catalyst is essential to both improve the liquefaction extent and decrease the liquefaction temperature and time. Generally, hydrochloric acid, phosphoric acid, and sulfuric acid could add as catalyst. For its higher acidity, sulfuric acid is mostly used. In an attempted research performed with substitution of 6% hydrochloric acid for sulfuric acid, the liquid yield was only 54% after 2 h; apparently, hydrochloric acid is not the appropriate catalyst [33].

Usually, the liquid yield decreases with an increase in the concentration of the catalyst in case of lower concentration of catalyst. When wheat straw was liquefied in the mixture of PEG 400 and glycerin, less of the residue per cent could be obtained in more catalyst. When the concentration of sulfuric acid increased from 1 to 5%, the residue per cent showed a decrease of about 70.8%. Due to the oxidizability and corrosion, 5% sulfuric acid was recommended [32]. In liquefaction of bagasse and cotton stalks using polyhydric alcohols (PEG 400 and glycerin), 3 and 4% acid concentrations were the more preferred concentrations. In the research, the recondensation reactions were not observed even at high acid concentration [31].

However, if the concentration of acid is in excess of a certain value, the recondensation reactions of the liquefied fragments are enhanced resulting in increase of insoluble residue. When bagasse was liquefied in EC, the residue content decreased drastically with an increase in the catalyst dosage during the initial stage, but the variations in the residue content were observed in the final stage when different amounts of the catalyst were used, the catalyst concentration in excess of 3% resulted in an increase in the residue content [34].

The role of the solvent is to dissolve liquefaction products and to prevent them from repolymerizing; the straw/solvent ratio (SSR) influences the liquefaction efficiency and the cost. Increase in SSR leads to more recondensation reactions

of the liquefied components, whereas the effect of solvent becomes unapparent and the cost becomes not affordable when the ratio decreases to a certain value. In wood liquefaction, most of the studies were focusing on liquefaction of wood at relatively small wood concentration, and the SSR was greater than 1/3. The optimum conditions of straws are different from that of wood because of their characteristics of physical and chemical properties.

For liquefaction of corn stover, SSR reached 1/3, the liquefaction yield was 91% after 2 h [33]. In liquefaction of bagasse and cotton stalks, the amount of residue decreased with changing SSR from 1/3 to 1/5. Applying low raw material concentration means higher capital investments, so moderate SSR 1/4 was recommended [31]. In liquefaction of wheat straw, the residue per cent decreased with an increase in SSR, and the recommended condition was SSR 1/6, with the residue being 13% [35].

The temperature for the liquefaction of straw with organic solution in the presence of catalyst is 120–160 °C, which is lower than the thermochemical liquefaction. At low temperature, the fibrous raw materials decomposed slowly, so the residue content decreased at a low speed. When the temperature increased, the decomposition was accelerated, and the residue content reduced drastically. However, a further increase in the temperature promoted the recondensation of the reaction intermediate, resulting in an increase in the residue content.

In liquefaction of wheat straw, the residue content decreased when the temperature decreased from 140 °C to lower, but the residue per cent increased when the temperature increased to 150 °C because of the recondensation [35]. The minimum residue content was obtained at 150 °C in liquefaction of bagasse, the residue content tended to increase when the temperature reached 160 °C, which was even higher than that at 140 °C [34]. In liquefaction of bagasse and cotton stalks, the residue remarkably decreased as the liquefaction temperature increased from 140 to 150 °C. Further increase in the liquefaction temperature to 180 °C resulted in very slight decrease in the amount of residue, but the recondensation were not observed even at high liquefaction temperature. Higher liquefaction temperatures were not necessary owing to the cost, and 150 °C was considered the suitable value [31].

In solvolytic liquefaction, free radical fragments formed in the degradation of lignin easily condense to form char in the presence of acid; the content of the free radical fragments increase with an increase in the reaction time. When the concentration of the degradation intermediates was high enough, recondensation occurred easily resulting in an increase in the residue content. Therefore, the reaction should be quenched in a certain time.

The residue content decreases rapidly during the initial stage of the reaction. This indicates that the straw components decompose and dissolve rapidly during this stage. The residue content tends to increase later, which suggests that the

recondensation of the degradation intermediates dominate the reaction at this time. Related research revealed that the reaction time should not exceed 18 [34] and 90 min [32] for bagasse in EC and wheat straw in mixture of PEG 400 and glycerin, respectively. In liquefaction of bagasse and cotton stalks, it was revealed that the rate of bagasse liquefaction was faster than that of cotton stalks. After 15 min, 26.9 and 35.8% remained as residue for bagasse and cotton stalks, respectively. Beyond that time, the percentage of residue decreased slowly till 6.6 and 9.1% after 105 min, but recondensation reaction was not observed at this long time [31].

The liquefaction products with low molecular weight are potential stuff for fuels or chemicals, and the liquefaction products possessing moderate molecular weight and active functional groups can be used to synthesize polymer material [32]. Polyol products with multiple hydroxyl groups produced from solvolytic liquefaction have been used to produce plastics, foams, resin, films, and adhesives [26].

Polyester was synthesized by cross-linking the liquefied corn stover with multifunctional carboxylic acids and/or cyclic acid anhydrides, succinic anhydride combined with additive, and polyethylene glycol. The tensile strength of polyester was about 5 MPa, and the elongation was around 35%, which was stable in cold water and organic solvents. The new biodegradable polymer could be used as sheets, films, and fibers in agriculture, gardens, packaging, and textile industries field [36].

Biodegradable rigid polyurethane foam (PUF) was produced from diisocyanate and liquefied wheat straw by mixing the liquefaction product, polyol TNR-410 with methylene diphenylene diisocyanate (MDI), catalyst Ditin *n*-butyl dilaurate (DBTDL), and foaming agent HCHC-141b, and other additives [32]. The compressive strength of PUF increased with increasing the content of liquefied wheat straw, the biodegradation was accelerated with increasing the content of liquefied wheat straw, and the thermal stability of PUF was improved by the addition of liquefied product. The apparent density, the resilience rate, the elongation, and the tensile strength of PUF based on liquefied wheat straw were comparable with those based on glycol, and the water absorption of PUF based on liquefaction was much higher than the others for the hydrophilic groups of 5-hydroxymethyl-2-furfural (HMF) and levulinic acid. Additionally, the weight loss of 16% in a year indicated that the PUF based on liquefied wheat straw had good biodegradability [35].

Epoxy resin with high adhesive shear strength and thermal stability was prepared from the liquefied bagasse, diglycidyl ether of bisphenol A, and triethylene tetramine. The maximum value adhesive shear strength of 19.00 MPa was achieved when the dosage of liquefied bagasse was 25%. The thermogravimetric analysis (TGA) curve of the novel resin had two regions representing the two steps of the thermal degradation, at 397.87 and 437.19 °C, respectively, compared with one step at 392.75 °C for the pure epoxy resin. It was

because the rigid aromatic structure of lignin derivatives restricted the motion of molecular chains and improved the thermal stability of epoxy resin. Additionally, the novel resin presented a higher  $T_g$  than the pure epoxy resin cured [34].

### 7.4.3 THERMAL GASIFICATION

Thermal gasification is a process in which feedstock reacts with gasifying agents in gasifier to produce syngas. Gasifying agents are  $O_2$ , air,  $CO_2$ , or their mixture, and syngas mainly consist of  $H_2$ ,  $CO$ ,  $CH_4$ , and  $CO_2$  [37]. Syngas having different calorific values (CV) could be produced by using different gasifying agents. Using air or air and steam mixture, the product gas has low CV ( $4-6 \text{ MJ/Nm}^3$ ); using  $O_2$  or steam, product gas has medium CV ( $12-18 \text{ MJ/Nm}^3$ ), whereas using  $H_2$  or hydrogenation, product gas with high CV ( $40 \text{ MJ/Nm}^3$ ) are obtained.

Gasification process involves complicated reaction occurring in the solid, liquid, and gas phases, and the main reactions are pyrolysis, partial oxidation, and steam gasification. Pyrolysis reaction processes without water or steam, the feedstock degrades into lower-molecular-weight products in gaseous, liquid, and solid forms; partial oxidation occurs with the amount of  $O_2$  less than that required for complete combustion in stoichiometric reaction; in steam gasification, the pyrolysis and partial oxidation products react with water to produce  $CO$ ,  $CO_2$ , and  $H_2$ ; and the water gas shift (WGS) reaction (water and  $CO$  react to form  $H_2$  and  $CO_2$ ) and methanation reaction occurs ( $CO$  and  $H_2$  react to form  $CH_4$  and  $H_2O$ ). More detail mechanisms have been reviewed in related article [38].

Evans and Milne classified the major reaction during the gasification process as three stages: primary, secondary, and tertiary stages [39, 40] (Fig. 7.4.3). In the primary stage,

feedstock forms low-molecular-weight product and charcoal. The low-molecular-weight products mainly include gaseous  $H_2O$ ,  $CO_2$ , and oxygenated vapors and primary oxygenated liquids derived from cellulose. In the secondary stage, the primary products in gas and liquid are converted to  $CO$ ,  $CO_2$ ,  $H_2$ ,  $H_2O$ , gaseous olefins, aromatics, and secondary condensed oils. The secondary reaction temperature is between  $700$  and  $850^\circ\text{C}$ . In the tertiary stages, when heating to  $850-1000^\circ\text{C}$ , part of secondary products condenses to form a liquid tertiary phase, mainly soot and coke.

Cellulose, hemicelluloses, and lignin are the major components of feedstock, and their thermal degradation has been studied extensively by TG analyzer. TGA studies were performed in inert atmosphere with low heating rates and low final temperature to prevent the secondary reactions and to get maximum char and combustible volatiles. In the research conducted by Williams and Besler [41], TGA analysis of wood and rice husks and samples of cellulose, hemicelluloses, and lignin were performed and the following observations were obtained: hemicelluloses represented by xylan decomposed at the temperature mainly between  $220$  and  $320^\circ\text{C}$ , cellulose decomposed at the temperature between  $250$  and  $360^\circ\text{C}$ , and lignin underwent gradual decomposition between  $180$  and  $500^\circ\text{C}$ . Hemicelluloses decomposed at the temperature between  $220$  and  $720^\circ\text{C}$  yielding char approximately 20% of the original hemicelluloses; cellulose decomposed at the temperature between  $250$  and  $720^\circ\text{C}$  producing char approximately 8% of the original cellulose; and lignin underwent gradual decomposition at temperature between  $80$  and  $500^\circ\text{C}$  and when heated to  $720^\circ\text{C}$ , it yielded 55% char of the original lignin. The inorganic components of the straw were converted into bottom ash including  $CaO$ ,  $K_2O$ ,  $P_2O_5$ ,  $MgO$ ,  $SiO_2$ ,  $SO_3$ , and  $Na_2O$ . Melt point of ash is around  $1000^\circ\text{C}$ , so operating

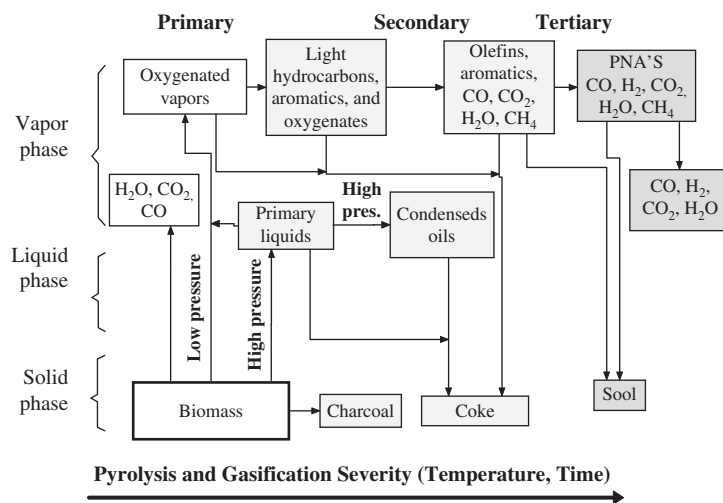


FIGURE 7.4.3 Gasification and pyrolysis reaction pathways in thermal gasification [40].



temperature should be maintained below this temperature to avoid ash sintering and slagging.

The gas composition outlet from the gasification reactor is dependent on the feedstock composition, the gasification process, and the gasifying agent, etc. Higher-molecular-weight hydrocarbons could condense to form tars leading to blockages and clogged filters. To decrease content of tar, catalysts are added into the gasification reactor. The chemical structure and formation of tars in feedstock gasification change with the temperatures. With an increase in the temperature, the content of mixed oxygenates, phenolic ethers, alkyl phenolic compounds, heterocyclic ethers, polyaromatic hydrocarbons, and larger polyaromatic hydrocarbons increases.

In gasification, heat and mass transfer are influenced by particle size of straw. Generally, the reaction rate increases with the decreasing of the particle size due to an increase in the specific surface area, whereas the reaction rate decreases with the decreasing of the particle size due to the decrease in porosity. However, in a certain scope, particle size of olive, grape bagasse [42], and *Cynara cardunculus L* [43] were between 0.4 and 2 mm; change of the main parameter of gasification efficiency were not obvious.

The reaction rate increases with an increase in temperature; higher temperature favors the pyrolysis and tar cracking reaction. Therefore, the yield of gas increases with an increment of temperature. In stream gasification of *Cynara cardunculus L*, the yield of gas increased from 0.56 at 650 °C to 0.88 at 800 °C, and correspondingly, the conversion also increased with an increase in the temperature, reaching 97.2% at 800 °C [43].

Air, pure O<sub>2</sub>, steam, CO<sub>2</sub>, or their mixtures are gasifying agents usually used. Syngas produced with air have low heat value because air contains a large amount of nitrogen. The heating value of syngas produced with pure O<sub>2</sub> is high, but the production of O<sub>2</sub> increases the overall cost. When steam was used as the gasifying agent, the heating value of the product gas was about 12–14 MJ/Nm<sup>3</sup> compared with 4–9 MJ/Nm<sup>3</sup> for gasification with air [44]. CO<sub>2</sub> was a promising gasifying agent, and the heating value and H<sub>2</sub> content of syngas can be increased when feedstock was gasified with CO<sub>2</sub> and with catalyst such as Ni/Al [45].

When gasification is carried out at high temperature, ash-related problems including sintering, agglomeration, deposition, erosion, and corrosion become obvious. Therefore, pretreatment is necessary to decrease the ash of straw for its high ash content compared with wood. Leaching and fractionation are the two main pretreatments. In fractionation pretreatment of peach stones for gasification, fractionation deteriorated the elemental composition of the ash of the peach stones, acted positively in decreasing the ash content of the feedstock and increasing of the amount of potassium in the ash of the feedstock [46], while leaching resulted in a significant removal of alkali metals and chlorine from the ash, and the residual ash had weak deposit size and strength in gasification. However, leaching had poor effect to wheat straw of removing

alkali metals, chlorine, and sulfur possibly because of the complex structure of straw [47].

The addition of catalyst could decrease the tar concentration. There are three groups of catalysts, which include (1) naturally occurring catalysts such as dolomite and olivine; (2) alkalis such as KOH, KHCO<sub>3</sub>, and K<sub>2</sub>CO<sub>3</sub>; (3) stable metals with oxide support such as nickel and alkali metals [48]. In gasification of sawdust, peanut shell, and wheat straw, three additives (dolomite, magnesite, and olivine) were added into the gasification process as catalyst for tar cracking. The results indicated that the addition of catalyst upgraded the gas product quality with tar removal efficiencies above 50% [49].

Fixed bed, moving bed, and fluidized bed gasifiers are the three types of gasifiers used in gasification. Fixed bed and moving bed gasifiers are simple and reliable designs and can gasify wet feedstock economically, but there are more tar and char because of nonuniform heat and mass transfer between solid and gas. In addition, the productivity is low in fixed bed. Fluidized bed gasifiers have been used widely in feedstock gasification for its high heating rate, uniform heating and high productivity, and lower char and tar contents in syngas. The overall gasification efficiency of rice husk in fluidized gasifier were higher than those in downdraft fixed bed gasifier: the productivity, gas heating value, and gasifier efficiency were 960 kg.m<sup>2</sup>/h, 4.6–6.3 MJ/Nm<sup>3</sup>, and 65% in the industrial-scale circulated fluidized bed gasifier, respectively, compared with 127 kg.m<sup>2</sup>/h, 3.8–4.6 MJ/Nm<sup>3</sup>, and 47% in the downdraft fixed bed gasifier [50].

The pressure and the equivalence ratio (ER, the ratio of O<sub>2</sub> required for gasification to O<sub>2</sub> required for full combustion of a given amount of feedstock) influence char and tar formulation. The value of ER is usually 0.2–0.4. In gasification of three feedstock samples (sawdust, peanut shell, and wheat straw) using a fluidized bed gasification reactor at 800 °C, with an increase of ER from 0.15 to 0.35, the gas yield increased rapidly from 1.14 to 1.93 m<sup>3</sup>/kg, whereas the lower heating value (LHV) of gas decreased from 7.09 to 3.26 MJ/m<sup>3</sup>. Meanwhile, methylbenzene and naphthalene increased with an increase in ER, whereas phenol and styrene production decreased [49].

The syngas can be used to generate heat and power, produce H<sub>2</sub>, and synthesize other chemicals and liquid fuels depending on the quality of syngas. Low heat value syngas from air gasification of feedstock can be fed to gas engines directly or gas turbines for power generation, and the advantage is that ash is not prone to slug. The heating value of the producer gas from gasification of cane trash at equivalence ratio ranging from 0.25 to 0.20 was in the range of 4.5–4.8 MJ/m<sup>3</sup> (dry gas), which was sufficient for stable gas turbine combustion [51]. Mixture of wood chips from short rotation forestry and forest and agricultural residues (olive stones and grape-seed flour) were gasified and were used to generate power in a plant [52]. The plant with the net power output of approximately 12 MWe, which was



operated in an atmospheric, air-blown, and circulating fluidized-bed (CFB) gasifier and integrated with a 10.9 MWe, single-shaft, heavy-duty gas turbine, was suitable to burn the low calorific value fuel gas.

Syngas could produce  $H_2$  in the reaction of water reforming and WGS reaction. In water reforming,  $CH_4$  react to  $H_2O$  to form  $H_2$  and  $CO$ ; and in WGS reaction,  $CO$  react to  $H_2O$  to  $H_2$  and  $CO_2$ ,  $CO_2$  can be removed from the syngas after gasification.

Switchgrass was converted into  $H_2$  fuel by gasification, steam reforming of tars, and reacting with  $CO$  in the producer gas [53]. Air-blown gasification of switchgrass produced relatively low concentrations of  $H_2$  (about 8.5 vol%). Steam reforming of tars and light hydrocarbons and reacting steam with  $CO$  via the WGS reaction increased the  $H_2$  content in the producer gas to 27.1 vol%. Degradation of the catalysts used in the steam reformer and WGS reactors occurred because of deposition of coke, sulfur, and chlorine on the catalysts.  $CO$  conversion in the high-temperature shift reactor reached 83%, whereas the overall conversion in the two-stage shift reaction system reached 98.7%. Syngas from discard seed corn could produce  $H_2$  through steam reform tar and WGS reaction, where  $CO$  and steam react to produce  $CO_2$  and  $H_2$  [54]. The catalytic tar reactor in combination with high temperature and low temperature WGS reactors increased  $H_2$  from 5.8 to 8.8 vol% to as high as 27–29 vol%, whereas the  $CO$  concentration of 13–15 vol% reduced to less than 0.5 vol%. The conversion of  $CO$  in the high temperature WGS reached 75 to 80%, whereas  $CO$  conversion by the combination of high temperature and low temperature WGS reactors exceeded 95%.

Methanol and dimethyl ether (DME) are the important chemicals used for fuels, and methanol could be produced in  $CO$  or  $CO_2$  hydrogenation in pretense of catalyst as follows:  $CO_2$  react to  $H_2$  to produce  $CH_3OH$  and  $H_2O$  [55], whereas DME can be produced by the dehydration of methanol.

Microorganisms could convert  $CO$  and  $H_2$  to multi-carbon compounds including organic acids, alcohols, and polyesters. Currently known syngas-fermenting bacteria, such as *Clostridium ljungdahlii*, *Clostridium autoethanogenum*, *Butyrivibrio methylotrophicum*, *Archaeoglobus fulgidus*, and so on, could convert the syngas to  $H_2$ , formate, acetate, ethanol, butyrate, and/or butanol, and the production of ethanol through fermentation by *Clostridium ljungdahlii* has been made commercial [56].

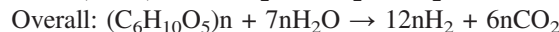
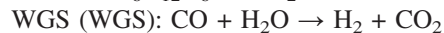
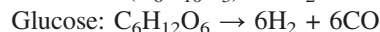
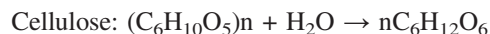
The mixture  $CO$  and  $H_2$  of syngas can be used to synthesize alkane with variable chain length via the Fischer Tropsch (FT) reaction, in which  $CO$  reacts with  $H_2$  to produce alkane and  $H_2O$  [57]. The obtained alkane is an alternative to conventional fuels such as diesel, kerosene, and gasoline. The syngas contains a large number of  $CH_4$  and  $CO_2$ , so steam reforming and water-shift reaction process is necessary to adjust the syngas to obtain the appropriate  $CO$  and  $H_2$  ratio, and the inert gas, such as  $CO_2$  and minim  $H_2S$ , should be removed as they are poisons to

catalyst. Compared with conventional fuels, alkane produced from feedstock contains less contaminants, such as sulfur and aromatics. Therefore, the prospect of feedstock alkane fuels is promising in the long run.

## 7.4.4 HYDROTHERMAL GASIFICATION

Gasification of feedstock by thermal methods involving partial oxidation to produce syngas, typically the moisture of feedstock, should be less than 10%. However, many of the feedstock resources are composed of material with higher levels of moisture, more typically 50% or more. To efficiently process such resources, novel technology gasification, hydrothermal gasification has been developed. Hydrothermal gasification is practiced in pressurized water (supercritical or subcritical state) over a range of operating temperatures and pressures. Apart from the suitability for feedstock with high water content, another advantage is a high gas yield and low tar and coke formation at relatively low temperatures.

The simplified reactions occurred in hydrothermal gasification are as follows [58]:

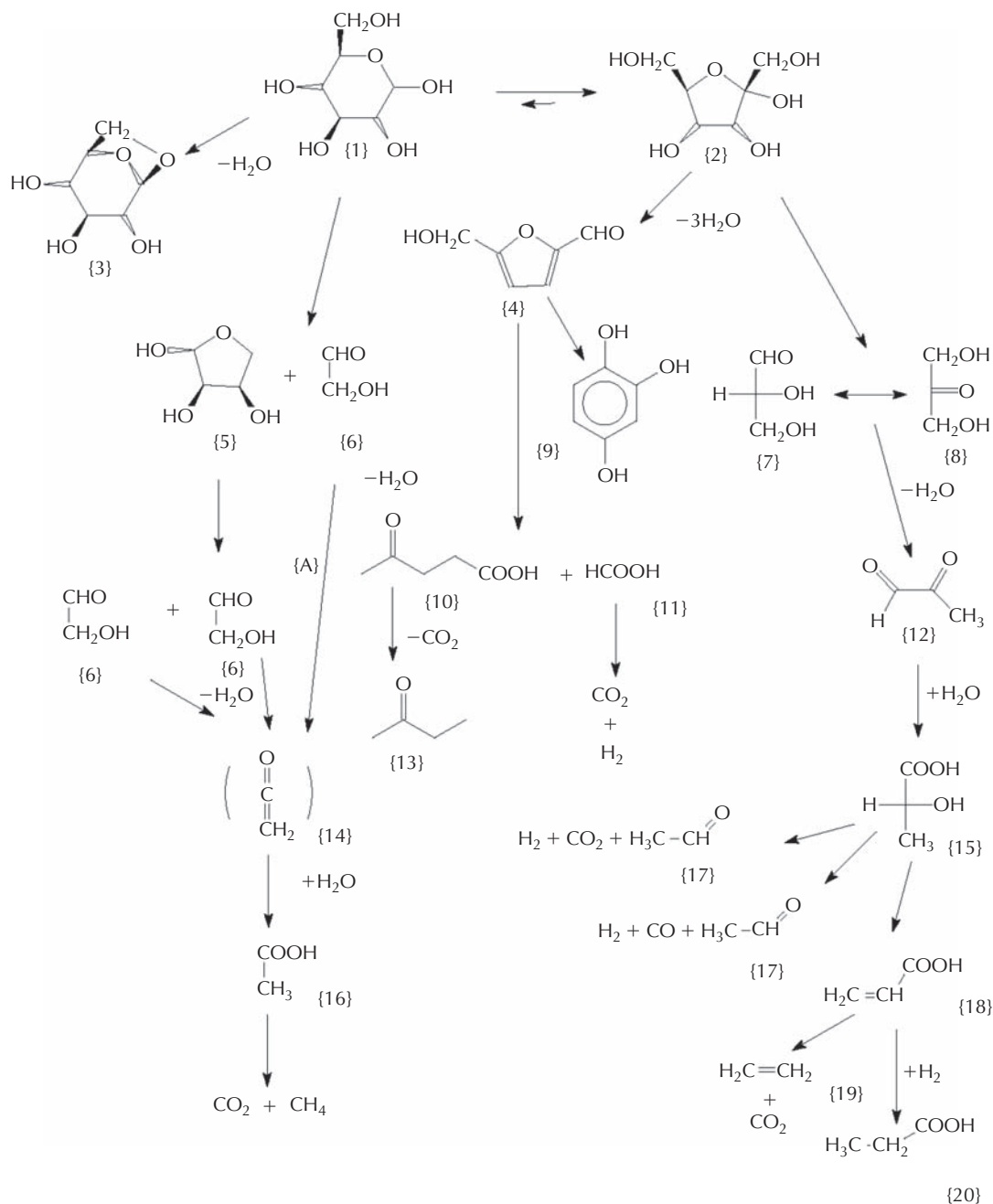


Hydrolysis of cellulose [59] and hemicelluloses [60] are rather rapid and complete in hydrothermal gasification, so their primary hydrolysis products are reasonable model compounds. Hydrolysis products degrade into intermediate and form gas and matter dissolved in water.

Water-soluble products, mainly sugars, were hydrolyzed products when the temperature was below 200 °C; water-soluble products, gas, oil, and cokes formed when the temperature was above 200 °C; when the temperature was higher than 300 °C, further decomposition occurred and more carbon were converted to coke and  $CO_2$  [61].

In hydrothermal degradation of cellulose between 300 and 400 °C, products were fructose, saccharinic acid, erythrose, glyceraldehydes, 1, 6-anhydroglucose, dihydroxyacetone, pyruvaldehyde, and small amounts of 5-hydroxymethylfurfural [62]. Williams et al. studied the composition of products after subcritical conversion of glucose and postulated that the reaction was Diels-Alder cyclo-addition reactions [63], and more detail knowledge about the degradation pathways of glucose and fructose and the formation of gases were summarized by Kruse [64] (Fig. 7.4.4).

Phenols are originated both from lignin and carbohydrate and are not easy to gasify. Phenols support the formation of a homogeneous phase during the conversion of the water and lignin mixture in high temperature and prevent polymerization. It is not easy to gasify lignins compared with carbohydrates, the degradation products of



**FIGURE 7.4.4** Reaction pathways of subcritical gasification glucose and fructose [64].

lignin gasification were CO, CO<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>, and char, and CH<sub>4</sub> formed from the hydrogenation of CO [65].

In a novel supercritical water gasification fluidized bed system for glucose and corncob, respectively,  $H_2$  yield decreased and  $CO$  yield increased as the feedstock concentration increased. With an increase in the solution concentration,  $CH_4$  yield increased for glucose gasification, whereas  $CH_4$  yield increased at low concentration and decreased at high concentration for corncob gasification [66]. Compared with glucose gasification, the molar of  $H_2$

was much lower, and the molar fraction of CO was much higher. The reason can be explained that more  $\text{K}_2\text{CO}_3$  content in corncob catalyzes the WGS reaction to produce  $\text{H}_2$  and  $\text{CO}_2$  instead of CO.

Catalysts are required for gas formation in hydrothermal gasification. Activated carbon catalyst was used to avoid char formation; alkali catalyst facilitates the WGS reaction in temperature between 500 and 700 °C; metal catalysts facilitate gasification in temperature between 374 and 500 °C [67]. During catalyst gasification of glucose, the

gas yield was increased by the addition of Raney nickel and  $K_2CO_3$  [68]. In supercritical water gasification of cotton stalk and corncob performed in a batch autoclave at 500 °C, catalysts, including  $K_2CO_3$ , Trona ( $NaHCO_3$ - $Na_2CO_3$ - $2H_2O$ ), red mud (Fe-oxide containing residue from Al-production), and Raney-Ni, enhance the WGS reaction and reform of  $CH_4$  producing more  $H_2$  [69].

The effect of catalyst on gasification varied with the type of feedstock. The gasification yield of corn starch was influenced by the addition of  $KHCO_3$ , whereas that of clover grass and corn silage, which contained approximately 1% potassium as salt, were not influenced by the addition [70]. Due to high amount of potassium in the sunflower stalk, the  $H_2$  yields did not increase with further addition of an alkali metal catalyst [69].

The effect of temperature on  $H_2$  production by feedstock gasification is complicated. In gasification of glucose in the presence of catalyst, both of the yield of  $H_2$  and that of  $CO_2$  increased significantly with an increase in temperature, but the yield of  $CH_4$  remained constant [71]. In gasification of corncob, the yield of  $H_2$  and  $CH_4$  increased from 21.78% to 38.42% with temperature increased from 550 to 660 °C, but that of CO decreased from 24.15 to 4.10% correspondingly. The reason can be explained that high temperature was in favor of free-radical reaction to produce gas. During catalyst gasification of glucose at 500 °C, the gas yield increased by high heating rate [68]. In gasification of sawdust and cornstarch in a tubular flow reactor at 685 °C, slower heating rates favored the formation of refractory compounds, resulting in more  $CH_4$  production, whereas the faster heating rates favored  $H_2$  formation [72].

Batch and tubular reactor are the two types of equipments for hydrothermal gasification. In tubular reactor, gas yield decreases with an increasing initial concentration of material [73], whereas in a continuously stirred tank reactor (CSTR, a batch type), the condition is opposite [74]. The reason is explained by kinetic approach: degradation of the intermediates to gases with low reaction order, and this reaction competes with the polymerization to tar and coke with high reaction order, so the relative gas yield in a tubular reactor decreases with initial concentration [64].

Mixtures of  $H_2$  and  $CH_4$  could mix into natural gas and make natural gas appear partially green. The mixture gas could be converted with WGS and steam reforming to decrease the hydrocarbons including  $CH_4$  to obtain purer  $H_2$  for fuel cell applications [61].

## REFERENCES

- [1] G.W. Huber, S. Iborra, A. Corma, Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering, *Chem. Rev.* 106 (2006) 4044–4098.
- [2] F. Behrendt, Y. Neubauer, M. Oevermann, B. Wilmes, N. Zobel, Direct liquefaction of biomass, *Chem. Eng. Technol.* 31 (2008) 667–677.
- [3] M. Balat, Mechanisms of thermochemical biomass conversion processes. Part 3: reactions of liquefaction, *Energy Sources Part A* 30 (2008) 649–659.
- [4] A. Demirbas, Mechanisms of liquefaction and pyrolysis reactions of biomass, *Energy Convers. Manage.* 41 (2000) 633–646.
- [5] A. Kruse, A. Gawlik, Biomass conversion in water at 330–410 °C and 30–50 MPa. Identification of key compounds for indicating different chemical reaction pathways, *Ind. Eng. Chem. Res.* 42 (2003) 267–279.
- [6] Z. Fang, T. Sato, R.L. Smith, H. Inomata, K. Arai, J.A. Kozinski, Reaction chemistry and phase behavior of lignin in high temperature and supercritical water, *Bioresour. Technol.* 99 (2008) 3424–3430.
- [7] Z. Fang, T. Minowa, R.L. Smith, T. Ogi, J.A. Kozinski, Liquefaction and gasification of cellulose with  $Na_2CO_3$  and Ni in subcritical water at 350 °C, *Ind. Eng. Chem. Res.* 43 (2004) 2454–2463.
- [8] A. Demirbas, Current technologies for the thermo conversion of biomass into fuels and chemicals, *Energy Sources* 26 (2004) 715–730.
- [9] B. Zhang, M. von Keitz, K. Valentas, Thermochemical liquefaction of high diversity grassland perennials, *J. Anal. Appl. Pyrolysis* 84 (2009) 18–24.
- [10] B. Zhang, M. von Keitz, K. Valentas, Thermal effects on hydrothermal biomass liquefaction, *Appl. Biochem. Biotechnol.* 147 (2008) 143–150.
- [11] H. Li, X. Yuan, G. Zeng, J. Tong, Y. Yan, H. Cao, L. Wang, M. Cheng, J. Zhang, D. Yang, Liquefaction of rice straw in sub- and supercritical 1,4 dioxane water mixture, *Fuel Process. Technol.* 90 (2009) 657–663.
- [12] C.B. Xu, T. Etcheverry, Hydro liquefaction of woody biomass in sub- and supercritical ethanol with iron based catalysts, *Fuel* 87 (2008) 335–345.
- [13] M. Cemek, M.M. Kucuk, Liquid products from *Verbascum* stalk by supercritical fluid extraction, *Energy Convers. Manage.* 42 (2001) 125–130.
- [14] M.M. Kucuk, S. Agirtas, Liquefaction of *Prangmites australis* by supercritical gas extraction, *Bioresour. Technol.* 69 (1999) 141–143.
- [15] F.M. Lancas, A.L. Rezemini, P.M. Donate, Upgrading of sugar cane bagasse by thermal processes. 9. Catalytic liquefaction in ethanol, *Energy Sources* 21 (1999) 299–308.
- [16] F.M. Lancas, P.A.R. Azocar, Upgrading of sugar cane bagasse by thermal processes. 8. Direct liquefaction with N alcohols, *Fuel Sci. Technol. Int.* 14 (1996) 979–992.
- [17] J.E. Miller, L. Evans, A. Littlewolf, D.E. Trudell, Batch microreactor studies of lignin and lignin model compound depolymerization by bases in alcohol solvents, *Fuel* 78 (1999) 1363–1366.
- [18] X.Z. Yuan, H. Li, G.M. Zeng, J.Y. Tong, W. Xie, Sub- and supercritical liquefaction of rice straw in the presence of ethanol water and 2-propanol water mixture, *Energy* 32 (2007) 2081–2088.
- [19] M.M. Kucuk, Liquefaction of biomass by supercritical gas extraction, *Energy Sources* 23 (2001) 363–368.
- [20] M. Erzenin, M.M. Kucuk, Liquefaction of sunflower stalk by using supercritical extraction, *Energy Convers. Manage.* 39 (1998) 1203–1206.
- [21] C.C. Song, H.Q. Hu, S.W. Zhu, G. Wang, G.H. Chen, Nonisothermal catalytic liquefaction of corn stalk in subcritical and supercritical water, *Energy Fuels* 18 (2004) 90–96.
- [22] A. Demirbas, Catalytic conversion of residual lignocellulosic materials to an acetone soluble oil, *Fuel Sci. Technol. Int.* 9 (1991) 425–433.

- [23] F. Yu, R. Ruan, P. Chen, S. Deng, Y. Liu, X. Lin, Liquefaction of corn cobs with supercritical water treatment, *Trans. ASABE* 50 (2007) 175 180.
- [24] J.E. Naber, F. Goudriaan, HTU® Diesel from biomass, ACS Division of Fuel Chemistry, 2005, <http://www.membership.acs.org/P/PETR/2005/Biorefineries/Presentation08.ppt>.
- [25] J.E. Naber, F. Goudriaan, Successfully using biomass to harness renewable energy in an efficient and cost effective way, 2004, <http://www.cpi.umist.ac.uk/eminent/Confidential/meeting/RigaMeeting/Riga%20Workshop/PresenatieHTUBiofuel.ppt>.
- [26] L.Y. Liang, Z.H. Mao, Y.B. Li, C.X. Wan, T.P. Wang, L.H. Zhang, L.Y. Zhang, Liquefaction of crop residues for polyol production, *Bioresources* 1 (2006) 248 256.
- [27] T. Yamada, Y. Hu, H. Ono, Condensation reaction of degraded lignocellulose during wood liquefaction in the presence of polyhydric alcohols, *J. Adhes. Soc. Jpn.* 37 (2001) 471 478.
- [28] T. Zhang, Studies on the Mild Liquefaction of Lignocellulosic Materials, Tsinghua University, Beijing, 2006.
- [29] T. Zhang, Y.J. Zhou, D.H. Liu, L. Petrus, Qualitative analysis of products formed during the acid catalyzed liquefaction of bagasse in ethylene glycol, *Bioresour. Technol.* 98 (2007) 1454 1459.
- [30] T. Yamada, H. Ono, Rapid liquefaction of lignocellulosic waste by using ethylene carbonate, *Bioresour. Technol.* 70 (1999) 61 67.
- [31] E.B.M. Hassan, N. Shukry, Polyhydric alcohol liquefaction of some lignocellulosic agricultural residues, *Ind. Crops Prod.* 27 (2008) 33 38.
- [32] F.G. Chen, Z.M. Lu, Liquefaction of wheat straw and preparation of rigid polyurethane foam from the liquefaction products, *J. Appl. Polym. Sci.* 111 (2009) 508 516.
- [33] F. Yu, R. Ruan, X.Y. Lin, Y.H. Liu, R. Fu, Y.H. Li, P. Chen, Y.Y. Gao, Reaction kinetics of stover liquefaction in recycled stover polyol, *Appl. Biochem. Biotechnol.* 130 (2006) 563 573.
- [34] T. Xie, F.G. Chen, Fast liquefaction of bagasse in ethylene carbonate and preparation of epoxy resin from the liquefied product, *J. Appl. Polym. Sci.* 98 (2005) 1961 1968.
- [35] H. Wang, H.Z. Chen, A novel method of utilizing the biomass resource: rapid liquefaction of wheat straw and preparation of biodegradable polyurethane foam (PUF), *J. Chin. Inst. Chem. Eng.* 38 (2007) 95 102.
- [36] F. Yu, Y.H. Liu, X.J. Pan, X.Y. Lin, C.M. Liu, P. Chen, R. Ruan, Liquefaction of corn stover and preparation of polyester from the liquefied polyol, *Appl. Biochem. Biotechnol.* 130 (2006) 574 585.
- [37] L. Wang, C.L. Weller, D.D. Jones, M.A. Hanna, Contemporary issues in thermal gasification of biomass and its application to electricity and fuel production, *Biomass Bioenergy* 32 (2008) 573 581.
- [38] V. Kirubakaran, V. Sivaramakrishnan, R. Nalini, T. Sekar, M. Premalatha, P. Subramanian, A review on gasification of biomass, *Renew. Sust. Energy Rev.* 13 (2009) 168 175.
- [39] R.J. Evans, T.A. Milne, Molecular characterization of the pyrolysis of biomass. 1. Fundamentals, *Energy Fuels* 1 (1987) 123 137.
- [40] T.A. Milne, R.J. Evans, N. Abatzoglou, Biomass gasifier tars: their nature, formation and conversion. Report No. NREL/TP 570 25357 National Renewable Energy Laboratory, Golden, CO, 1998.
- [41] P.T. Williams, S. Besler, The pyrolysis of rice husks in a thermogravimetric analyzer and static batch reactor, *Fuel* 72 (1993) 151 159.
- [42] J.M. Encinar, F.J. Beltran, A. Bernalte, A. Ramiro, J.F. Gonzalez, Pyrolysis of two agricultural residues: olive and grape bagasse, influence of particle size and temperature, *Biomass Bioenergy* 11 (1996) 397 409.
- [43] J.M. Encinar, J.F. Gonzalez, J. Gonzalez, Steam gasification of *Cynara cardunculus* L.: influence of variables, *Fuel Process. Technol.* 75 (2002) 27 43.
- [44] J. Gil, J. Corella, M.P. Aznar, M.A. Caballero, Biomass gasification in atmospheric and bubbling fluidized bed: effect of the type of gasifying agent on the product distribution, *Biomass Bioenergy* 17 (1999) 389 403.
- [45] L. Garcia, M.L. Salvador, J. Arauzo, R. Bilbao, CO<sub>2</sub> as a gasifying agent for gas production from pine sawdust at low temperatures using a Ni/Al coprecipitated catalyst, *Fuel Process. Technol.* 69 (2001) 157 174.
- [46] S. Arvelakis, H. Gehrman, M. Beckmann, E.G. Koukios, Preliminary results on the ash behavior of peach stones during fluidized bed gasification: evaluation of fractionation and leaching as pre treatments, *Biomass Bioenergy* 28 (2005) 331 338.
- [47] S. Arvelakis, P. Vourliotis, E. Kakaras, E.G. Koukios, Effect of leaching on the ash behavior of wheat straw and olive residue during fluidized bed combustion, *Biomass Bioenergy* 20 (2001) 459 470.
- [48] D. Sutton, B. Kelleher, J.R.H. Ross, Review of literature on catalysts for biomass gasification, *Fuel Process. Technol.* 73 (2001) 155 173.
- [49] H.P. Chen, B. Li, H.P. Yang, G.L. Yang, S.H. Zhang, Experimental investigation of biomass gasification in a fluidized bed reactor, *Energy Fuels* 22 (2008) 3493 3498.
- [50] X.L. Yin, C.Z. Wu, S.P. Zheng, Y. Chen, Design and operation of a CFB gasification and power generation system for rice husk, *Biomass Bioenergy* 23 (2002) 181 187.
- [51] M. Gabra, E. Pettersson, R. Backman, B. Kjellstrom, Evaluation of cyclone gasifier performance for gasification of sugar cane residue Part 2: gasification of cane trash, *Biomass Bioenergy* 21 (2001) 371 380.
- [52] H.J. De Lange, P. Barbucci, The THERMIE energy farm project, *Biomass Bioenergy* 15 (1998) 219 224.
- [53] R.Q. Zhang, R.C. Brown, A. Suby, Thermochemical generation of hydrogen from switchgrass, *Energy Fuels* 18 (2004) 251 256.
- [54] R.Q. Zhang, K. Cummer, A. Suby, R.C. Brown, Biomass derived hydrogen from an air blown gasifier, *Fuel Process. Technol.* 86 (2005) 861 874.
- [55] A. Demirbas, Biomethanol production from organic waste materials, *Energy Sources A* 30 (2008) 565 572.
- [56] A.M. Henstra, J. Sipma, A. Rinze, A.J.M. Stams, Microbiology of synthesis gas fermentation for biofuel production, *Curr. Opin. Biotechnol.* 18 (2007) 200 206.
- [57] M.J.A. Tijmensen, A.P.C. Faaij, C.N. Hamelinck, M.R.M. Van Hardeveld, Exploration of the possibilities for production of Fischer Tropsch liquids and power via biomass gasification, *Biomass Bioenergy* 23 (2002) 129 152.
- [58] Z. Fang, T. Minowa, C. Fang, R.L. Smith, H. Inomata, J.A. Kozinski, Catalytic hydrothermal gasification of cellulose and glucose, *Int. J. Hydrogen Energy* 33 (2008) 981 990.
- [59] Y. Ogihara, R.L. Smith, H. Inomata, K. Arai, Direct observation of cellulose dissolution in subcritical and supercritical water over a wide range of water densities (550 1000 kg/m<sup>3</sup>), *Cellulose* 12 (2005) 595 606.
- [60] W.S.L. Mok, M.J. Antal, Uncatalyzed solvolysis of whole biomass hemicellulose by hot compressed liquid water, *Ind. Eng. Chem. Res.* 31 (1992) 1157 1161.

- [61] Y. Matsumura, T. Minowa, B. Potic, S.R.A. Kersten, W. Prins, W.P.M. van Swaaij, B. van de Beld, D.C. Elliott, G.G. Neuenschwander, A. Kruse, M.J. Antal, Biomass gasification in near and supercritical water: status and prospects, *Biomass Bioenergy* 29 (2005) 269–292.
- [62] B.M. Kabyemela, T. Adschiri, R.M. Malaluan, K. Arai, Kinetics of glucose epimerization and decomposition in subcritical and supercritical water, *Ind. Eng. Chem. Res.* 36 (1997) 1552–1558.
- [63] P.T. Williams, J. Onwudili, Subcritical and supercritical water gasification of cellulose, starch, glucose, and biomass waste, *Energy Fuels* 20 (2006) 1259–1265.
- [64] A. Kruse, Supercritical water gasification, *Biofuels Bioprod. Biorefin.* 2 (2008) 415–437.
- [65] F.L.P. Resende, S.A. Fraley, M.J. Berger, P.E. Savage, Noncatalytic gasification of lignin in supercritical water, *Energy Fuels* 22 (2008) 1328–1334.
- [66] Y.J. Lu, H. Jin, L.J. Guo, X.M. Zhang, C.Q. Cao, X. Guo, Hydrogen production by biomass gasification in supercritical water with a fluidized bed reactor, *Int. J. Hydrogen Energy* 33 (2008) 6066–6075.
- [67] D.C. Elliott, Catalytic hydrothermal gasification of biomass, *Biofuels Bioprod. Biorefin.* 2 (2008) 254–265.
- [68] A. Sinag, A. Kruse, J. Rathert, Influence of the heating rate and the type of catalyst on the formation of key intermediates and on the generation of gases during hydropyrolysis of glucose in supercritical water in a batch reactor, *Ind. Eng. Chem. Res.* 43 (2004) 502–508.
- [69] J. Yanik, S. Ebale, A. Kruse, M. Saglam, M. Yuksel, Biomass gasification in supercritical water: II. effect of catalyst, *Int. J. Hydrogen Energy* 33 (2008) 4520–4526.
- [70] P. D'Jesus, N. Boukis, B. Kraushaar Czarnetzki, E. Dinjus, Gasification of corn and clover grass in supercritical water, *Fuel* 85 (2006) 1032–1038.
- [71] P. Azadi, A.A. Khodadadi, Y. Mortazavi, R. Farnood, Hydrothermal gasification of glucose using Raney nickel and homogeneous organometallic catalysts, *Fuel Process. Technol.* 90 (2009) 145–151.
- [72] M.J. Antal, S.G. Allen, D. Schulman, X.D. Xu, R.J. Divilio, Biomass gasification in supercritical water, *Ind. Eng. Chem. Res.* 39 (2000) 4040–4053.
- [73] P. D'Jesus, C. Artiel, N. Boukis, B. Kraushaar Czarnetzki, E. Dinjus, Influence of educt preparation on gasification of corn silage in supercritical water, *Ind. Eng. Chem. Res.* 44 (2005) 9071–9077.
- [74] A. Kruse, T. Henningsen, A. Sinag, J. Pfeiffer, Biomass gasification in supercritical water: influence of the dry matter content and the formation of phenols, *Ind. Eng. Chem. Res.* 42 (2003) 3711–3717.



# Biorefinery Straw for Bioethanol

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## 7.5.1 INTRODUCTION

The 20th century was marked by dramatic changes in energy supply. During this period, the dominant energy supply shifted from biomass to coal in about 1900 (or early 20th century), and then shifted from coal to oil in about 1950 (or mid 20th century). There are strong indications that equally and potentially more dramatic changes in energy supply will happen in the 21st century, particularly in the case of oil [1]. The matter of when the world oil supply will peak and enter into a period of permanent decline is a matter of considerable controversy [2, 3]. Meanwhile, during the past 150 years, human activities have caused a dramatic increase in the emission of greenhouse gases, e.g. CO<sub>2</sub>, which has led to changes in the equilibrium of the earth's atmosphere. The content of CO<sub>2</sub> in the air has increased from 280 to 365 ppm during this period [4]. Therefore, the increased concern for the security of the oil supply and the negative impact of fossil fuels on the environment has put pressure on society to find renewable fuel alternatives. The European Commission plans to substitute progressively 20% of conventional fossil fuels with alternative fuels in the transport sector by 2020, with an intermittent goal set at 5.75% in 2010, and one-fourth of the EU's transportation fuels will be derived from biofuels by 2030 [5]. In the United States, the Energy Policy Act of 2005 requires blending of 7.5 billion gallons of alternative fuels by 2012, and the former president George W. Bush had set the goal to reduce America's dependence on foreign sources of oil by replacing more than 75% of oil imports by 2025 [6].

It should be pointed out that the use of bioethanol as a source of energy would be more than just complementing for solar, wind, and other intermittent renewable energy sources in the long run [7]. In 1925, Henry Ford had quoted ethanol as "the fuel of the future." Now, ethanol has already been introduced on a large scale in many countries, and it is expected to be one of the dominating renewable biofuels in

the transport sector within the coming 20 years [8]. Ethanol can be blended with petrol and used as neat alcohol. Compared with single gasoline, ethanol has a higher octane number (96–113) that reduces the need for toxic, octane-enhancing additives. It is also a provider of oxygen, which helps to reduce the emission of carbon monoxide (CO), nitrogen oxides (NO<sub>x</sub>), noncombusted hydrocarbon, and reduces the exhaust of volatile organic compounds after combustion [9–11]. In addition, ethanol is about 15% more efficient than gasoline in optimized spark-ignition engines [12]. Thus, with only about two-thirds of the volumetric energy content of gasoline, ethanol can still be able to drive 75–80% of the distance on a given volume of ethanol [10]. Furthermore, the production of ethanol by fermentation offers a more favorable trade balance, enhanced energy security, and a major new crop for a depressed agricultural economy. Ethanol also reduces smog formation because of its low volatility and photochemical reactivity, its combustion products are low, and only low levels of smog-producing compounds are formed by its combustion [13]. Today, all cars with a catalyst can be run on a mixture of 90% gasoline and 10% ethanol without adjusting the engine. New cars can even use mixtures containing up to 20% ethanol. There are also new engines available that can run on pure ethanol, and the so-called flexible fuel vehicles are able to use mixtures of 0–85% ethanol in gasoline (E85).

In 2004, the world ethanol production was estimated to be 72 billion liters [14], and the quantity will pass 90 billion liters in 2012. The United States has now overtaken Brazil as the largest producer of bioethanol in the world, with annual production of nearly 3 billion gallons. There are currently more than 100 U.S. bioethanol production facilities, with many more under construction and in the planning stages. The number of E85 bioethanol fuel stations in the United States has also grown significantly to 700. About 655 million bushels of corn were utilized in the fuel ethanol industry and that accounted for 92% of the

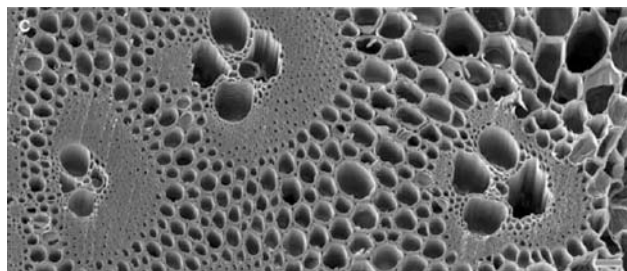
feedstocks in 2001 [15]. The increase of ethanol production from corn will supplement the corn-based food and feed production. Brazil stands out as a pioneer in the use of bioethanol as a road transport fuel: its experience of bioethanol-powered cars began before the World War II. Brazil had been the world's leader (and primary user) of fuel ethanol for more than 25 years, producing slightly less than half the world's total in 2004. In 2006, the number of bioethanol-powered cars on Brazilian roads hit the 2 million mark, and flex-fuel cars accounted for more than three-quarters of the nation's new car sales. Now, all fuelling stations in Brazil sell pure (95%) ethanol (E95) and gasohol, a 25% ethanol/75% gasoline blend (E25). Brazil's huge supply of surplus sugarcane crops is the basis for its robust bioethanol industry. Fuel ethanol production is considerably more modest in the European Union, where France, Spain, and Sweden are the three largest producers. The EU25 amounted to 2040 ktOE in 2004 or about 0.7% of the market. As a strategic move taken by the Chinese government to promote sustainable economic and social development and environmental protection, China had gained a yearly output of bioethanol gasoline of 10.2 million tons in 2006, accounting for 20% of its overall gasoline consumption, and unfolded a trial use of bioethanol gasoline (a mixture of 10% ethanol and 90% gasoline) in five provinces and 27 cities. According to the plan for the coming 5 years, China will build four major manufacturing plants for bioethanol with yield capacity of about 1 million tons per year, listed third in the world.

The amount of solar energy received at the earth's surface is  $2.5 \times 10^{21}$  Btu/year, which far exceeds the present human usage of  $2.0 \times 10^{17}$  Btu/year. The amount of energy from the sun that is stored as carbon via photosynthesis is 10 times the world usage. The traditional feedstocks such as molasses, sugarcane juice, corn, etc. being used for current ethanol production have social and economic barriers. Besides these feedstocks, lignocellulosic biomass such as cereal straws, which is the most abundant biomass on earth [16], is an alternative feedstock for bioethanol production. It is inexpensive, plentiful, and renewable. On a worldwide basis, terrestrial plants produce  $1.3 \times 10^{10}$  metric tons (dry weight basis) of wood per year, which is equivalent to  $7 \times 10^9$  metric tons of coal or about two-thirds of the

world's energy requirement. Available cellulosic feedstocks from agriculture and other sources are about 180 million tons per year. Furthermore, tremendous amounts of cellulose are available as municipal and industrial wastes, which currently contribute to our pollution problems. This biomass, including forest residues such as wood; agricultural residues such as sugarcane bagasse, corn cob, corn stover, wheat, and rice straw; industrial residues such as pulp and paper processing waste and municipal solid wastes; and energy crops such as switch grass, is the most promising potential feedstock for fuel ethanol [9, 16, 17]. According to Kim and Dale's research [18], the total potential bioethanol production from crop residues and wasted crops is 491 GL/year, about 16 times higher than the current world ethanol production. In particular, rice straw, 731 Tg/year, potentially results in about 205 GL bioethanol; wheat straw, 354 Tg/year, potentially results in about 104 GL bioethanol; corn stover 203.6 Tg/year, potentially results in about 58.6 GL bioethanol; sugarcane bagasse, 108 Tg/year, potentially results in about 51 GL bioethanol; barley straw, 120 Tg/year, potentially results in about 18 GL bioethanol; oat straw, 11 Tg/year, potentially results in about 1.8 GL bioethanol; sorghum straw, 10.3 Tg/year, potentially results in about 1.8 GL bioethanol. The potential bioethanol production can replace 353 GL of gasoline, which is equivalent to 32% of the total worldwide consumption of gasoline, when bioethanol is used in E85 for a midsize passenger vehicle. Thus, the use of lignocellulosic biomass as a renewable source for liquid fuel production is of great research interest.

## 7.5.2 LIGNOCELLULOSIC BIOMASS RECALCITRANCE

Lignocellulosic biomass such as cereal straw is often described as "recalcitrance" because of its complex structural and chemical mechanisms for resisting assault on its structural sugars from the microbial and animal kingdoms (Fig. 7.5.1) [19]. Natural factors believed to contribute to the recalcitrance of lignocellulosic feedstock to chemicals or enzymes include the following: (i) the epidermal tissue, particularly the cuticle and epicuticular

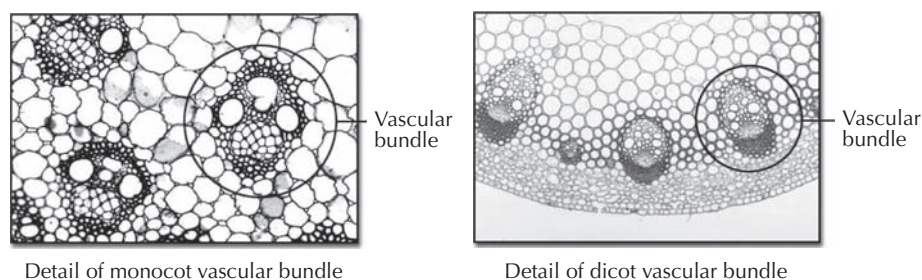


**FIGURE 7.5.1** A scanning electron micrograph of the cross section of a maize stem shows vascular bundles and pith tissues, as well as the diverse cell sizes, shapes, and cell wall thicknesses typical for higher plant structure. Scale bar, 50  $\mu$ m [19].

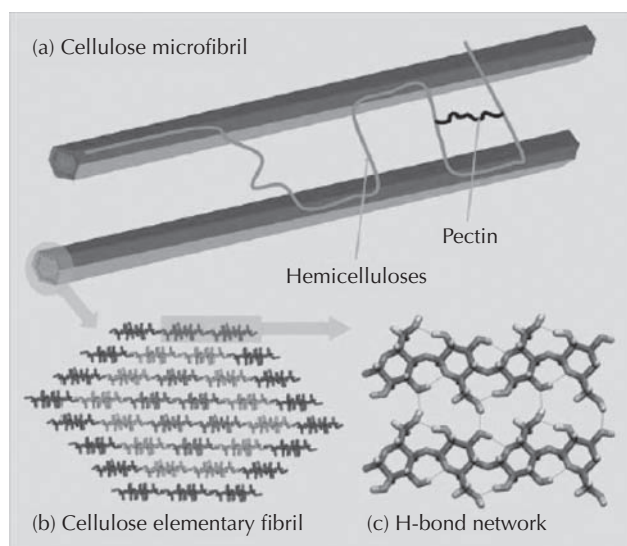
waxes, and dense cells of the plant body forming the rind of grasses and bark of trees; (ii) the arrangement and density of the vascular bundles both in monocot and dicot plants that carefully limit liquid penetration throughout plant stems (Fig. 7.5.2) [20]; (iii) the relative amount of sclerenchymatous (thick wall) tissue; (iv) the composite nature of the plant cell wall that restricts aqueous penetration from cell to cell; (v) the structural heterogeneity and complexity of cell-wall constituents such as microfibrils and matrix polymers [21]; (vi) the crystalline nature of cellulose itself; and (vii) the inherent difficulty for enzymes acting on an insoluble substrate [22]. Meanwhile, some “process-induced” causes of recalcitrance should not be ignored. For example, (i) high mechanical pressure, such as that from plug feeders, collapses the natural vascular structure; (ii) dilute-acid chemical pretreatments may permit cellulose to reanneal, leading to “hornification” of cellulose in

microfibers, and (iii) some pretreatment may permit lignin to become soluble and “plate out” on cellulose surfaces during the cool-down phase; (iv) the inhibitors to subsequent fermentations that exist naturally in cell walls or are generated during conversion processes [23]. In the context of the biorefinery, these chemical and structural features of biomass affect liquid penetration and/or enzyme accessibility and activity and, thus, conversion costs.

At the molecular level (Fig. 7.5.3) [19], the crystalline cellulose core of cell-wall microfibrils [24] is highly resistant to chemical and biological hydrolysis because of its structure, in which chains of cellobioses are precisely arranged. The chair conformation of the glucose residues in cellulose forces the hydroxyl groups into radial (equatorial) orientation and the aliphatic hydrogen atoms into axial positions. As a result, there is strong interchain hydrogen bonding between adjacent chains in a cellulose sheet and



**FIGURE 7.5.2** Vascular bundle structures in the monocot and dicot plant, respectively [20]. (see color plate)



**FIGURE 7.5.3** (a) A simplified model showing the interaction of the major polysaccharides in the cell wall. (Lignin is not shown here because its interactions are not well established.) In this system, hemicelluloses are closely associated to the surface of the rigid cellulose crystallite forming the microfibril network. Pectins are cross linked polysaccharides forming a hydrated gel that “glues” the cell wall components together. (b) The 36 chain model of the cellulose elementary fibril. Here, the depiction of the glucan chains is based generally on an X ray structure of cellulose I $\beta$ . It has been proposed that the cellulose elementary fibril may contain three groups of glucan chains: in group C1 (red), there are six true crystalline chains; in group C2 (green), there are 12 subcrystalline chains with a small degree of disorder; and in group C3 (blue), there are 18 surface chains that are subcrystalline with a large degree of disorder. (c) The intra and interchain hydrogen bond network in cellulose I $\beta$  [19]. (see color plate)

weaker hydrophobic interactions between cellulose sheets. The hydrophobic face of cellulose sheets makes crystalline cellulose resistant to acid hydrolysis because it contributes to the formation of a dense layer of water near the hydrated cellulose surface. The strong interchain hydrogen-bonding network makes crystalline cellulose resistant to enzymatic hydrolysis, whereas hemicelluloses and amorphous cellulose are readily digestible. Higher-order structures in plants also contribute to biomass recalcitrance. For example, access to the crystalline cellulose cores of microfibrils is restricted by a coating of amorphous cellulose and hemicelluloses. At a microscopic and macroscopic scale, the complex heterogeneous nature of biomass creates mass-transport limitations for delivery of chemical or biochemical catalysts. Effectively overcoming the recalcitrance structure of lignocellulose and releasing the locked polysaccharides is one of the most important and urgent R&D priorities for the emerging cellulosic ethanol and biobased chemical industries.

### 7.5.3 BIOREFINERY STRAW FOR BIOETHANOL PRODUCTION

The emerging concept of a sustainable green biorefinery is strongly cross-disciplinary. It is based on a renaissance of biomass used as feedstock for fuels, chemicals, and materials along with several key developments in science and engineering. The biorefinery concept is analogous to today's petroleum refineries, which produce multiple fuels and products from petroleum. Industrial biorefineries have been identified as the most promising route to the creation of a new domestic biobased industry. By producing multiple products, a biorefinery can take advantage of the differences in biomass components and intermediates and maximize the value derived from the biomass feedstock (Fig. 7.5.4) [25]. A biorefinery might, for example, produce one or several low volume, but high-value, chemical products and a low value, but high-volume liquid transportation fuel. In the mean while, it might generate

electricity and process heat for its own use and perhaps enough for sale of electricity. The high-value products enhance profitability, the high-volume fuel helps meet national energy needs, and the power production reduces costs and avoids greenhouse-gas emissions. Lignocellulose biorefineries via biological conversion generally have three main steps: (1) lignocellulose pretreatment, which converts the recalcitrant lignocellulose structure to reactive cellulosic intermediates; (2) enzymatic cellulose hydrolysis, by which cellulases hydrolyze reactive intermediates to fermentable sugars (e.g., glucose and xylose); and (3) fermentation, which produces cellulosic ethanol or other biobased chemicals (e.g., lactic acid, succinic acid). Acid hydrolysis as one of the oldest methods used in saccharification of lignocellulosic biomass is also mentioned in this chapter.

#### 7.5.3.1 Acid Hydrolysis

Acid hydrolysis of plant lignocellulosic biomass has been known since 1819 [26]. Examples are the modified Bergius process (40% HCl) operated during World War II in Germany, and the more recently modified Scholler processes (0.4%  $\text{H}_2\text{SO}_4$ ) in the former Soviet Union, Japan, and Brazil [27]. Although sulfuric acid is the most investigated acid, some researchers also used nitric acid and phosphoric acid at varying concentration, reaction time, and temperature to hydrolyze the sugar cane bagasse. Hydrochloric acid was found to be less effective for degradation of xylose than sulfuric acid. Acid hydrolysis can be divided into two groups: (i) concentrated-acid hydrolysis; and (ii) dilute-acid hydrolysis. A comparison between concentrated- and dilute-acid hydrolysis methods is presented in Table 7.5.1.

##### 7.5.3.1.1 Kinetic Study

The kinetic model of cellulosic hydrolysis by sulfuric acid that involves two consecutive first-order reactions was firstly developed by Saeman [28]:

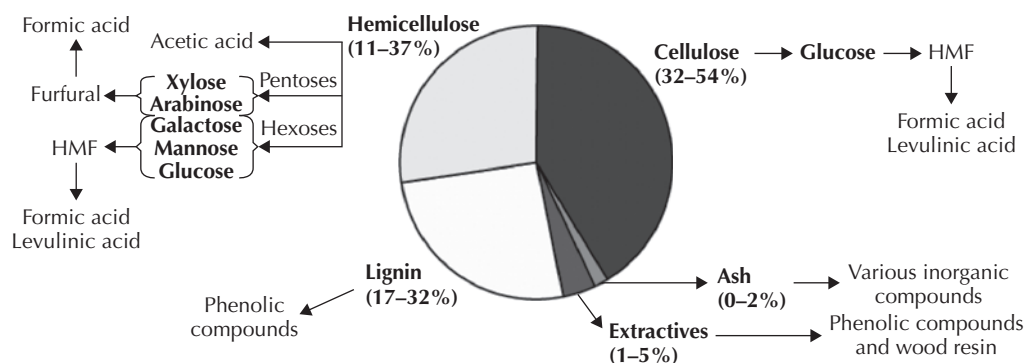


FIGURE 7.5.4 Composition of lignocellulosic materials and their potential biofinery products [25].



**TABLE 7.5.1** Comparison Between Concentrated- and Dilute-Acid Hydrolysis Methods

Hydrolysis methods	Advantages	Disadvantages
Concentrated acid process	1. Operated at low temperature 2. High sugar yield	1. High acid consumption 2. Equipment corrosion 3. High energy consumption for acid recovery 4. Longer reaction time (2–6 h)
Dilute acid process	1. Low acid consumption 2. Short residence time	1. Operated at high temperature 2. Low sugar yield 3. Equipment corrosion 4. Formation of undesirable byproduct

Recently, some researchers [29, 30] applied this model to other polysaccharides, such as xylan, mannan, arabinan, etc., which can be generalized as:



where  $k_1$  and  $k_2$  are the first-order reaction rate constants for monomer release and decomposition, respectively, both having units of reciprocal time. The polymers can be cellulose, hemicelluloses; monomers can be glucose, xylose, arabinose, etc.; and decomposition products can be furfural, hydroxymethylfurfural, formic acid, levulinic acid, etc. On the basis of the assumption of the isothermal batch reactor and the negligible initial monomer concentration, the following equation is obtained for the concentration of monomers:

$$M = P_0 \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \quad (7.5.3)$$

where  $P_0$  is the initial concentration of polymers and  $M$  is the concentration of monomers released at reaction time  $t$ . Assuming the reaction rate constant,  $k_1$ , has Arrhenius type temperature dependence, it can be calculated as following equation:

$$k_1 = A_1 \exp \left[ -\frac{E_1}{RT_1} \right] \quad (7.5.4)$$

where  $E_1$  is the activation energy (kJ/mol),  $R = 8.1343 \times 10^3$  (kJ/mol/K),  $T_1$  is the temperature (K), and  $A_1$  is the preexponential factor (per minute).

Lignocellulosic biomass can be categorized in a fast fraction or slow fraction based on the rate of hydrolysis of hemicelluloses and cellulose components. Rodríguez-Chong et al. [29] and Gamez et al. [30] found the two-fraction model for such type of polymers fitted better. The two fractions are related to a parameter  $a$ , the mass fraction of the susceptible polymer in the raw material. The simplest case of the two-fraction model happens when the less susceptible fraction does not involve in any reaction and

always remains in the solid phase. For this condition, the kinetic equation for the concentration of monomers is as follows [30]:

$$M = P_0 a \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \quad (7.5.5)$$

### 7.5.3.1.2 Concentrated Acid Hydrolysis

The concentrated acid hydrolysis process for producing sugars and ethanol from lignocellulosic biomass has existed for a long time. The native cellulose in cotton was found able to be dissolved by concentrated sulfuric acid followed with water dilution in a literature as early as 1883 [31]. The processes involving concentrated acids are operated at low temperature and give high sugar yields, over 90% recoveries of both glucose and xylose were achieved [32]. These processes typically involve the use of 60–90% sulfuric acid, mild temperature, and moderate pressures created by pumping materials from one vessel to another for effective hydrolysis. The concentrated acid disrupts the hydrogen bonding between cellulose chains, and converts cellulose to a completely amorphous state. Once cellulose has been decrystallized, it forms a homogeneous gel with the acid [33]. In Arkenol's process, decrystallization is carried out by adding 70–77% of sulfuric acid to rice straw that has been dried to 10% moisture. Acid is added at a ratio of 1.25:1 (acid: cellulose + hemicelluloses), and temperature is controlled at less than 80 °C. Diluting the acid to 20–30% with water and heating the solution at 100 °C for an hour result in the release of sugars. The gel from this reactor is pressed to remove an acid/sugar product stream. Residual solids are subjected to a second hydrolysis step [33]. At least 98% of sugar was recovered by a chromatographic separation technique with less than 3% loss of acid which was recycled for the hydrolysis step [34]. Iraj et al. [35] performed the concentrated acid hydrolysis of mixed wood chips and found that maximum sugar recovery (78–82% of



theoretical yields) was achieved at sulfuric acid concentration of 26% for 2 h of residence time.

However, as the concentration of acid is very high (30–70%) in this method, the dilution and heating of the concentrated acid during the hydrolysis process make it extremely corrosive, toxic, and hazardous. Therefore, the process requires either expensive alloys or specialized nonmetallic constructions, such as ceramic or carbon-brick lining. In addition, when sulfuric acid is used, the neutralization process produces large amounts of gypsum and the recycling process makes it economically unfeasible [36, 37]. Despite the disadvantages, the concentrated-acid process is still of interest. It has recently been exploited by a Dutch research group in a process called “Biosulfurol” [38]. The process was claimed to have a low overall cost for the ethanol produced. Furthermore, two full-scale cellulose-to-ethanol projects are under development in North America by Arkenol Inc. [39] (Mission Viejo, Calif.) and Masada Resource Group [40] (Birmingham, Ala.) based on concentrated-acid hydrolysis. Arkenol holds a series of patents on the use of concentrated acid to produce ethanol. They are currently working with U.S. Department of Energy (DOE) to establish a commercial facility that will convert rice straw to ethanol. The economics of this opportunity are driven by the availability of a cheap feedstock that normally poses a disposal problem. Arkenol’s technology further improves the economics of raw straw conversion by allowing for the recovery and purification of silica present in the straw.

### 7.5.3.1.3 Dilute-Acid Hydrolysis

Dilute-acid hydrolysis has been successfully developed for the hydrolysis of lignocellulosic material. The first established dilute-acid hydrolysis process was probably the Scholler process [41]. This was a batch process, in which the wood or straw material was kept in 0.5% sulfuric acid at 11–12 bar for approximately 45 min. In general, acid hydrolysis of lignocellulose is conducted with mineral acids such as dilute  $\text{H}_2\text{SO}_4$  and  $\text{HCl}$  in the range of 2–5%, at temperatures of about 160 °C and pressures of about 10 atm [42]. It is especially useful for the conversion of polysaccharides in hemicelluloses to monomeric sugars (arabinose, galactose, glucose, mannose, and xylose) and oligosaccharide that can be further fermented to ethanol by many microorganisms. The maximum yield of glucose is obtained at high temperature and short residence time, but even under these conditions the glucose yield is only between 50% and 60% of the theoretical value [10]. Because of the higher temperature and pressure, the hemicellulosic derived monosaccharides will degrade and give rise to fermentation inhibitors such as furan compounds, weak carboxylic acids, and phenolic compounds [43–45]. Removal of these compounds leads to the additional costs. The use of lime to neutralize acid has the

disadvantage of significant loss of sugar in the gypsum. However, such processes could be replaced by highly economical chromatographic separations with acid recycling. Therefore, one-step mild dilute-acid hydrolysis is usually used as one of the pretreatment methods for further enzymatic hydrolysis, and it will be discussed in the next section (Section 3.2.1.2, Dilute-acid prehydrolysis).

To avoid degradation of monosaccharides at high temperature and formation of the inhibitors, dilute-acid hydrolysis is carried out in two stages. In the first stage, hydrolysis is carried out under relatively mild conditions. This enables the second acid hydrolysis step to proceed under harsher conditions without degrading the hemicellulose sugars to inhibitor products. In the second stage, the residual solid is hydrolyzed under more severe conditions, allowing cellulose to be hydrolyzed. Karimi et al. [46] reported the dilute-acid hydrolysis of rice straw. The hydrolyses were carried out in a 10-L reactor, where the hydrolysis retention time (3–10 min), pressure (10–35 bar), and sulfuric acid concentration (0–1%) were examined. For the second stage of hydrolysis, the best results were achieved at the hydrolysis pressure of 30 bar and the retention time of 3 min, where a total of 78.9% of xylan and 46.6% of glucan were converted to xylose and glucose, respectively. Sanchez et al. [47] carried out the two-stage dilute-acid hydrolysis using Bolivian straw material, *Paja brava* (*P. brava*). In first stage, *P. brava* material was pretreated with steam followed by dilute sulfuric acid (0.5 or 1.0% by wt) hydrolysis at temperatures between 170 and 230 °C for a residence time between 3 and 10 min. The highest yield of hemicelluloses derived sugars were achieved at a temperature of 190 °C, and a reaction time of 5–10 min, whereas in second-stage hydrolysis considerably higher temperature (230 °C) was found good for hydrolysis of remaining fraction of cellulose.

### 7.5.3.2 Enzymatic Hydrolysis

In nature, various cellulolytic microorganisms produce enzymes that can either function synergistically and associate with the microorganism (such as the cellulosome) [48, 49] or act independently (such as most fungal and many bacterial cellulases). Although it is not fully known how many enzymes are involved in cell-wall deconstruction, three general categories of enzymes are considered necessary to hydrolyze native cell-wall material: cellulases, hemicellulases, and the accessory enzymes, which include hemicelluloses debranching, phenolic acid esterase, and possibly lignin degrading and modifying enzymes [50]. The process based on enzymatic hydrolysis and fermentation (Fig. 7.5.5) is currently regarded as the most promising alternative way of converting the carbohydrates in lignocellulosic materials into ethanol with high yields and low production cost [4, 51].

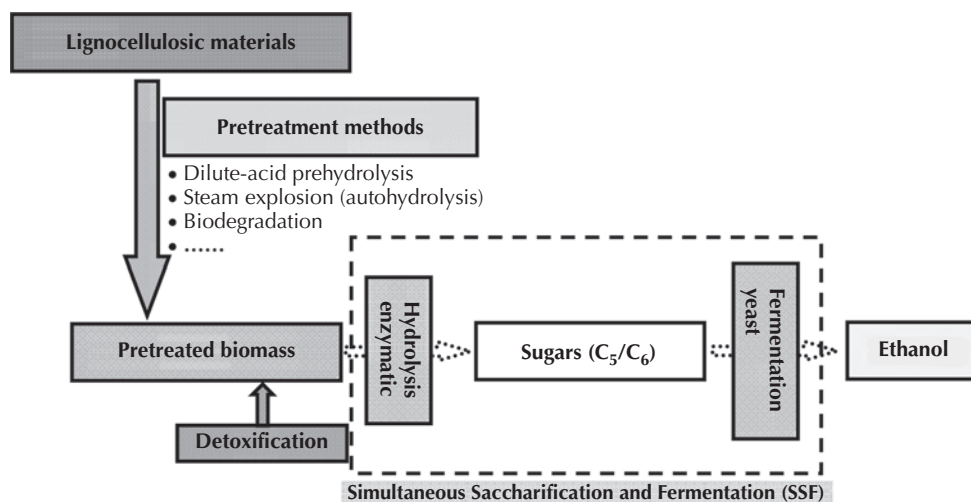


FIGURE 7.5.5 Process diagram of bioethanol production.

### 7.5.3.2.1 Pretreatment

Although both chemical and enzymatic processes have been studied extensively for the bioconversion of lignocellulose, enzymatic-based processes are more preferred over the last 20 years. This may be attributed to a number of factors, including improved understanding of the catalytic enzymes combined with decreased production costs, and the high specificity that enzymatic processes endow a conversion process. However, hydrolysis of cellulose to glucose in aqueous media catalyzed by the cellulase enzyme suffers from slow reaction rates, because the highly crystalline structure of cellulose makes the penetration of enzymes to the active sites very difficult, and the protective function from the matrix of hemicelluloses and lignin also makes the active sites hardly accessible. Consequently, substrate accessibility is of paramount importance to bioconversion via enzymatic processes, and any physical or chemical barrier restricting access for the enzyme can result in decreased hydrolysis. Accordingly, various pretreatment processes are investigated to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic chemical composition and structure so that hydrolysis of carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields. The pretreatment reduces crystallinity and increases the available surface area and pore volume of the water-insoluble, cellulose-rich component, and releases most of the hemicelluloses to the water phase. Recent researches have clearly proved that there is a direct correlation between the removal of lignin and hemicelluloses and the digestibility of cellulose [52]. Theoretically, it is possible to fractionate the carbohydrates of any species into solution, release the majority of the hemicelluloses into solution, and leave the cellulose fraction intact [53]. The pretreatment process is one of the most expensive steps and has a major influence on the costs of both prior and subsequent operations.

A number of lignocellulose pretreatment technologies are under intensive investigation on both laboratory scale and as pilot plants. The major pretreatment processes with potential for industrial application are steam explosion (autohydrolysis), dilute-acid prehydrolysis, and biological pretreatment.

#### 7.5.3.2.1.1 Steam Explosion (Autohydrolysis)

Steam explosion with or without catalyst (inorganic acid) is one of the popular pretreatment methods of lignocellulosic materials. In this method, chipped biomass is treated with high temperature/pressure saturated steam (170–280 °C), for relatively short residence times (10 s to as long as 20 min). Physical and chemical changes of the lignocellulosic materials take place during the treatment, probably due to the hydrolysis and solubilization of the hemicelluloses component, partial solubilization of the cellulose, and modification of lignin. Lignin is only poorly solubilized [54], although it may undergo extensive modification including condensation [55] and redistribution following liquefaction and coalescence under the heat of saturated steaming [56]. This process of “lignin melting” may contribute to opening up the lignocellulosic, but condensation and localization of the lignin to the matrix surface may have negative consequences by blocking. After the saturated steam treatment, the pressure is swiftly reduced, which makes the material to undergo an explosive decompression. This is generally thought to improve enzymatic hydrolysis of the resulting pulp, due to mechanical shearing and defibrillation of the fiber, and associated increase in accessible surface area. The major advantages of steam explosion pretreatment are that (1) it produces a lignocellulose material that is quite susceptible to enzymatic hydrolysis, (2) its energy requirements are considerably less than mechanical process, and (3) it does not have the recycling or environmental costs associated with

**TABLE 7.5.2** Pretreatment Conditions for Steam Exploded Lignocellulosic Materials

No.	Substrate	Steam explosion conditions			Ref.
		Temperature (°C)	Time (min)	Catalyst	
1	Barley straw	200 220	1.2 5		[59]
2	Barley straw	190 200	10	2% SO <sub>2</sub>	[60]
3	Barley straw	210	5	1% H <sub>2</sub> SO <sub>4</sub>	[61]
		190	5	2% H <sub>2</sub> SO <sub>4</sub>	
4	Rice straw	74	20	AFEX	[62]
5	Rice straw	225 251	0.5 10		[63]
6	Rice straw	170	30		[64]
7	Wheat straw	312	1	0.08 N H <sub>2</sub> SO <sub>4</sub>	[65]
8	Wheat straw	200 220	3 33		[66]
9	Wheat straw	205 230	2		[67]
10	Wheat straw	198	10		[68]
11	Wheat straw	205 230 (batch reactor)	2 10		[69]
		193 225 (continuous reactor)	2 6		
12	Wheat straw	160 200	5 20	0.9% H <sub>2</sub> SO <sub>4</sub>	[70]
13	Wheat straw	200 220	1.2 5		[59]
14	Wheat straw	190 210	2 10	0.2% H <sub>2</sub> SO <sub>4</sub>	[71]
15	Wheat straw sorghum bagasse	190	8		[72]
		210	2		
16	Oat straw	200 220	1.2 5		[59]
17	Sugarcane bagasse	188 243	0.5 44		[73]
18	Sugarcane bagasse	170 230	1 46		[74]
19	Corn stover	190	5	3% SO <sub>2</sub>	[75]
20	Corn stover	160 190	1.2 14	1% H <sub>2</sub> SO <sub>4</sub>	[76]
21	Corn stover	180 200	5	0 3% H <sub>2</sub> SO <sub>4</sub>	[77]
22	Corn stover	200	5	2% H <sub>2</sub> SO <sub>4</sub>	[78]
23	Corn stover	90	5	AFEX	[79]
24	Corn stover	170	15	15% Ammonia	[80]
25	Eel grass	160 180	1 5	0 2% H <sub>2</sub> C <sub>2</sub> O <sub>4</sub>	[81]

predominantly chemical pretreatments [57, 58]. The steam explosion pretreatment of lignocellulosic residuals, including straws, corn stover, sugar bagasse, and grass for enzymatic process are summarized in Table 7.5.2. The limitations of steam explosion include destruction of a portion of the xylan fraction, incomplete destruction of the lignin carbohydrate matrix, and generation of compounds

that may be inhibitory to microorganisms used in downstream processes.

During steam explosion pretreatment, there are two main process variables: temperature and time. To maximize recovery for each fraction of pretreated materials, Overend et al. [82] have proposed the introduction of a single factor, the reaction ordinate  $R_0$  that allows for the evaluation of the

explosion process. This factor is based on the assumption that overall process follows first-order reaction kinetics, and increases as a function of time  $t$  (min) and temperature  $T$  (°C) as follows:

$$R_0 = te^{(T-100)/14.75}$$

Chum et al. [83] introduced a third parameter, the environmental pH, into the aforementioned equation to describe the combined severity (CS):

$$CS = \log R_0 - \text{pH}$$

where pH is calculated from the amount of sulfuric acid added.

Addition of  $\text{H}_2\text{SO}_4$  (or  $\text{SO}_2$ ) in steam explosion can effectively improve enzymatic hydrolysis, decrease the production of inhibitory compounds, and lead to more complete removal of hemicelluloses. The  $\text{SO}_2$  itself is probably not the actual catalyst in the steam explosion, instead its sulfuric acid either formed by oxidation or disproportion inside wood cells may take the function, preferably inside wood cells. Other possible explanation suggest that  $\text{SO}_2$  may act as a weak acid by simply dissolving in water inherent in the green wood chips to form sulfurous acid, and it may selectively attack the wood lignin to form lignosulfonates which are very strong acids; or it may act in both these ways depending upon the level of  $\text{SO}_2$  impregnation [84]. Ammonia fiber explosion (AFEX), which seems like another kind of explosion pretreatment process, can also significantly improve the enzymatic hydrolysis. As the function of catalyst ammonia, hemicelluloses components are not obviously soluble, and some types of inhibitory by-products, such as furans, are not formed. However, to reduce the cost and protect the environment,  $\text{H}_2\text{SO}_4$  and ammonia must be recycled after the pretreatment.

#### 7.5.3.2.1.2 Dilute-Acid Prehydrolysis

Dilute-acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. The dilute-acid pretreatment takes function through hydrolyzing glycosidic bonds in hemicelluloses, lignin-hemicelluloses bonds, and perhaps also the lignin bonds in the pretreatment. This leads to degradation of hemicellulosic sugars into monomeric sugars and soluble oligomers and an increase in porosity of the plant cell walls, which makes the cellulose fibers more accessible to cellulase enzymes and achieves high reaction rates [85]. Compared to other pretreatment, dilute-acid prehydrolysis is especially useful for the conversion of xylan in hemicelluloses to xylose that can be further fermented to ethanol by many microorganisms [86].

The typical reaction conditions of hardwood and herbaceous biomass reported in the literature were as follows: a sulfuric acid catalyst in water with concentration of 0.5–1.0% (v/v); a temperature between 140 and 160 °C;

and the reaction times of 0–60 min at 140 °C, and 0–20 min at 160 °C. Xylose yields generally ranged from 70 to 95%. The lignin produced from the dilute-acid process is more condensed than that produced by the steam explosion process [52]. Sun and Cheng [87] pretreated rye straw and bermudagrass with dilute sulfuric acid (0.6–1.5%, w/w) at 121 °C for 30–90 min. About 50–66% of xylan in the biomass was hydrolyzed into monomeric xylose, and the glucose yield in the range of 30–52% and 46–81% of the theoretical potential was obtained for rye straw and bermudagrass, respectively. Grohmann et al. [88] reported the dilute sulfuric acid pretreatment of wheat straw. About 80% of xylan was removed at 140 °C for 1 h and enzymatic digestibility of cellulose was nearly 80%. Torget et al. [89] investigated the dilute sulfuric acid pretreatment of herbaceous crops, and they found that about 92% of the xylan was solubilized and 75% enzymatic digestibility of cellulose was obtained when switchgrass and weeping lovegrass were pretreated by 0.5% (v/v) sulfuric acid at 140 °C for 60 min and 160 °C for 10 min. Additionally, corn cobs and corn stover were pretreated with diluted sulfuric acid (0.45–0.5%, v/v) at 160 °C for 5–10 min, and more than 90% xylan was solubilized [89]. Zhao et al. [90] compared three different kinds of chemical pretreatment methods (sulfuric acid, sodium hydroxide, and peracetic acid), and concluded that peracetic acid pretreatment seemed to be the most effective method for improving enzymatic saccharification of the weed stem. The conversion ratio of cellulose under the optimal condition (90 °C, 150 min, loading of chemical of 50% based on raw materials and liquid-to-solid ratio of 4:1) was increased to 50% by cellulase loading of 80 FPU/g cellulose for 72 h incubation. The maximum yield of monomeric sugars from wheat straw (7.83%, w/v) by dilute  $\text{H}_2\text{SO}_4$  (0.75%, v/v) pretreatment and enzymatic saccharification (45 °C, pH 5.0, 72 h) using cellulase,  $\beta$ -glucosidase, xylanase, and esterase was  $565 \pm 10$  mg/g. Under this condition, no measurable quantities of furfural and hydroxymethyl furfural were produced. The yield of ethanol prepared from the enzymatic saccharification of acid pretreated wheat straw hydrolyzate by recombinant *Escherichia coli* strain FBR5 was 0.24 g/g [91]. Under the condition of 1%  $\text{H}_2\text{SO}_4$  and 27 min reaction time, rice straw was prehydrolyzed at 121 °C, and yielded 77% of xylan in the hydrolyzates [92].

The main drawback of dilute-acid prehydrolysis processes is the degradation of the sugars in hydrolysis reactions and formation of undesired by-products. The decomposition lowers the yield of sugars, and the undesired by-products severely inhibit the formation of ethanol during the fermentation process. Furthermore, the cost of dilute-acid pretreatment is usually higher than physicochemical pretreatment processes such as steam explosion or AFEX. A neutralization of pH is necessary for the downstream enzymatic hydrolysis and fermentation.

### 7.5.3.2.1.3 Biological Pretreatment

Biological pretreatment of agricultural residues is a new method for improvement of digestibility [93]. The organisms predominantly responsible for lignocellulose degradation are fungi, and the most rapid degraders in this group are basidiomycetes [94, 95]. In this process, microorganisms such as brown-, white-, and soft-rot fungi are used to degrade lignin and hemicelluloses in waste material. Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. The most widely studied white-rot organism is *P. chrysosporium*, which is one of the *holobasidiomycetes*. *Trichoderma reesei* and its mutants are the most studied ascomycete fungi, and these are used for the commercial production of hemicellulases and cellulases [96, 97]. The ability to degrade lignocellulosic materials efficiently is considered to be associated with a mycelial growth habit that allows the fungus to transport scarce nutrients such as nitrogen and iron, to a distance into the nutrient-poor lignocellulosic substrate that constitutes its carbon source. Fungal pretreatment lowers the energy requirements and chemical consumption of thermomechanical processes of lignocellulosic biomass, and could potentially be applied to enzyme-based biomass conversion processes. Recent studies showed that several microorganisms had been isolated and identified as biodegraded organisms on lignocellulosic residues (Table 7.5.3).

The digestibility of agricultural residues is strongly dependent on the types of their fibers. The biodegradation

of plant residues was pea > barley > wheat > rice > wood, concerning with the lignin, C/N ratio and N contents [108, 109]. Jale et al. [93] found that 9/13 species of *Basidiomycetes* significantly enhanced the digestibility of wheat straw. Hemicelluloses showed the largest proportionate loss, whereas lignin showed the least loss. Hatakka [108] also studied the pretreatment of wheat straw by 19 white-rot fungi and found that 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* in 5 weeks. Similar conversion was obtained in the pretreatment by *Phanerochaete sordida* 37 and *Pycnoporus cinnabarinus* 115 in 4 weeks. The colonization of maize (*Zea mays* L.) and rice (*Oriza sativa* L.) straw for 15 and 30 days by three fungi and a cellulaseless mutant of *P. chrysosporium* were investigated for dry matter (DM) digestibility. It was found that *Cyathus stercoreus* improved the in vitro cellulose digestibility of maize and rice straw by 37 and 45%, respectively, and DM loss was only increased to 3.3%. The wild and mutant strains of *Phanerochaete* degraded both hemicelluloses and cellulose indiscriminately. No direct correlation was found between lignin degradation and improved digestibility [110]. Akin et al. [111] reported that the biodegradation of Bermuda grass stems by ruminal microorganisms, after treatment for 6 weeks with *Ceriporiopsis subvermispora* or *Cyathus stercoreus*, was improved by 29–32% and 63–77%, respectively; dry weight losses caused by pretreatment with the fungi were about 20% more than that of untreated, control stems.

**TABLE 7.5.3 Fungus Used in Biodegradation of Several Agricultural Residues**

Substrates	Fungus	Ref.
Hay and straw	<i>Aspergillus terreus</i>	[98]
Cotton straw	<i>Pleutotus ostreatus</i>	[99]
Corn stover	<i>Cyathus stercoreus</i>	[100]
Sorghum stover	<i>Pleurotus sajor caju</i>	[101]
Wheat bran	<i>Pleurotus ostreatus</i>	[102]
Wheat straw	<i>Trametes versicolor</i> <i>Lentinus crinitus</i>	[103]
Sugarcane bagasse	<i>Trametes sp.</i> <i>Aspergillus niger</i> <i>Pleurotus ostreatus</i>	[104]
Barley bran	<i>Phanerochaete chrysosporium</i> <i>Trametes versicolor</i>	[105]
Wheat straw	<i>Pleutotus ostreatus</i> <i>Pleutotus pulmonarius</i>	[106]
Wheat and rice straw	<i>Bacillus polymyxa</i>	[107]



### 7.5.3.2 Detoxification Process

Contrary to sucrose- and starch-based ethanol production, lignocellulose-based production is a mixed-sugar fermentation in the presence of inhibiting compounds. As a function of the variable of conditions and methods of pretreatment (mainly dilute-acid prehydrolysis), different inhibitors may dominate in terms of concentrations. However, the observed inhibition is determined by a combined effect of several substances [112]. Furthermore, the quantitatively dominant inhibitors are not the only factor to determine the fermentability of a hydrolyzate. The toxicity of a hydrolyzate is found to differ from that of a synthetic medium with the same amount of the major hydrolyzate inhibitors added, indicating the importance of other inhibitors present in trace amounts [113].

Acetic acid, formic acid, and levulinic acid are the most common carboxylic acids found in the hydrolyzates. Acetic acid is mainly formed from acetylated sugars in the hemicelluloses, which are cleaved off already under mild pretreatment condition, reached to even higher than 10 g/l [114]. Furfural and hydroxymethylfurfural (HMF) are the only furans usually found in hydrolyzates in significant amounts. Furfural has been found to inhibit the in-vitro activity of aldolase, phosphofructokinase, triosephosphate dehydrogenase, and alcohol dehydrogenase. Xylose was found to be more sensitive to the acidity and high temperature conditions, and it is easily decomposed to furfural, while glucose is more resistant to harsh conditions [115]. HMF is not as toxic to *Saccharomyces cerevisiae* as furfural; however, HMF remains much longer than furfural in the medium, and consequently, the effects of HMF last longer than those of furfural. A large number of phenolic/aromatic compounds have been detected in dilute-acid hydrolyzates [116]. These compounds are believed to be degradation products of lignin during the hydrolysis. Moreover, they may also originate from sugar degradation and are present in lignocelluloses as extractives. The concentrations of the phenolic/aromatic compounds are normally a few milligrams per liter, probably due to the low water solubility of many of the phenolic compounds, or because of a limited degradation of lignin during the hydrolysis process. Among the phenolic compounds, the less heavily substituted phenolics are probably the most inhibitive materials in the hydrolyzates [112, 117].

A number of detoxification methods, including biological, physical, and chemical ones, have been proposed to transform inhibitors into inactive compounds or to reduce their concentration. An overview of the present applications of several detoxification processes in the hydrolyzate from wood materials is listed in Table 7.5.4. In comparison, the applications of several detoxification methods in the hydrolyzate from wood materials are also present in Table 7.5.4. The effectiveness of a detoxification method depends both on the type of hemicelluloses hydrolyzate and on the

species of microorganism employed, because each type of hydrolyzate has a different degree of toxicity, and each species of microorganism has a different degree of tolerance to inhibitors [118]. In this case, the fermentation productivity will be a function of the inhibitor concentration, the conversion capacity of the yeast and the quality of the process control. Just recently, it was demonstrated that the overexpression of a gene encoding alcohol dehydrogenase activity improved the fermentation of a 5-hydroxymethyl furfural-containing medium [119].

### 7.5.3.2.3 Enzymatic Hydrolysis

#### 7.5.3.2.3.1 Kinetic Study

Kadam et al. [132] developed a multireaction kinetic model for closed-system enzymatic hydrolysis of lignocellulosic biomass. Three hydrolysis reactions were modeled: two heterogeneous reactions for breakdown of cellulose to cellobiose and glucose, and one homogeneous reaction for hydrolyzing cellobiose to glucose. It has been found that the various mass transfer steps are quite fast with very low resistance, and the overall hydrolysis rate is mainly controlled by the surface kinetics promoted by the adsorbed enzyme. It has also been reported that the initial hydrolysis rate strongly depends on the initial extent of soluble protein adsorption and the effectiveness of the adsorbed soluble protein to promote hydrolysis.

The absorption of cellulase enzymes on the lignocellulosic residue and cellulose must be taken into account in the development of hydrolysis kinetics. The equilibrium adsorption of an enzyme on cellulose and on the lignocellulosic residue could be represented by the Langmuir-type isotherm:

$$[E]_{ad} = \frac{[A]_{max}[S]_{total}[E]_{free}}{K_d + [E]_{free}} \quad (7.5.6)$$

where  $[E]_{ad}$  is the concentration of adsorbed cellulase (mg cellulase/mL);  $[E]_{free}$  is the concentration of cellulase in solution (mg cellulase/mL);  $[S]_{total}$  is the total substrate concentration (mg cellulose/mL); and  $K_d$  is an equilibrium constant (mg/mL).

The initial hydrolysis rate,  $v_0$ , can be expressed as a function of the initial enzyme concentration,  $[E_0]$ . Accordingly, it is convenient to define a maximal velocity,  $V_{max}$ , and a corresponding half saturation constant,  $K_e$ , represented by the Michaelis-Menten-type kinetics:

$$v_0 = \frac{V_{max}[E_0]}{K_e + [E_0]} \quad (7.5.7)$$

With an increment in the concentration of products, such as cellobiose and glucose in enzymatic hydrolysis, the enzyme activity is inhibited. The inhibition pattern depends on the cellulase binding constant, enzyme concentration, maximum adsorption of the enzyme (cellulose surface area

**TABLE 7.5.4** Detoxification Processes for Improving the Yield of Bioethanol

Substrates	Pretreatment method	Detoxification process	Ref.
Corn straw	Steam explosion	Drying and water washing	[120]
Rice straw	Steam explosion	Activated charcoal	[121]
Sorghum straw	Acid hydrolysis	Activated charcoal	[122]
Corn cob	Acid hydrolysis	Overliming with Ca(OH) <sub>2</sub>	[123]
Sugarcane bagasse	Acid hydrolysis	Electrodialysis	[124]
Sugarcane bagasse	Acid hydrolysis	Overliming with Ca(OH) <sub>2</sub> , activated charcoal and laccase treatment	[125]
Wheat straw	Acid hydrolysis	Overliming with Ca(OH) <sub>2</sub> and ether extraction	[126]
Wheat straw	Acid hydrolysis	Overliming with Ca(OH) <sub>2</sub>	[91]
Sugarcane bagasse	Acid hydrolysis	CaO, H <sub>3</sub> PO <sub>4</sub> and activated charcoal	[127]
Sugarcane bagasse	Acid hydrolysis	Ion exchange resins and acidified activated charcoal	[128]
Spruce	Acid hydrolysis	Ion exchange resins	[129]
Poplar wood	Steam explosion	Water, ethyl acetate water extraction, and Ca(OH) <sub>2</sub>	[130]
Willow	Steam and SO <sub>2</sub>	Laccase and peroxidase from the white rot fungus	[131]

accessible to the enzyme), the range of substrate concentration, and  $\beta$ -glucosidase activity. The rate equations based on a competitive mode of sugar inhibition are developed using the following assumptions: (1) the enzyme adsorption follows a Langmuir-type isotherm with the first-order reactions occurring on the cellulose surface; (2) the cellulose matrix is uniform in terms of its susceptibility to enzymatic attack; (3) the enzyme activity remains constant; and (4) the conversion of cellobiose to glucose occurs in solution and follows the classical Michaelis-Menten kinetics [132].

The cellulose-to-cellobiose reaction with competitive glucose, cellobiose, and xylose inhibition can be written as

$$r_1 = \frac{k_{1r}E_{1B}R_sS}{1 + \frac{G_2}{K_{11G1}} + \frac{G}{K_{11G}} + \frac{X}{K_{11X}}} \quad (7.5.8)$$

The cellulose-to-glucose reaction with competitive glucose, cellobiose, and xylose inhibition can be written as:

$$r_2 = \frac{k_{2r}(E_{1B} + E_{2B})R_sS}{1 + \frac{G_2}{K_{21G2}} + \frac{G}{K_{21G}} + \frac{X}{K_{21X}}} \quad (7.5.9)$$

The cellobiose-to-glucose reaction with competitive glucose and xylose inhibition can be written as:

$$r_3 = \frac{k_{3r}E_{1F}G_2}{K_{3M}\left(1 + \frac{G}{K_{31G}} + \frac{X}{K_{31X}}\right) + G_2} \quad (7.5.10)$$

where  $r_1$ ,  $r_2$ , and  $r_3$  are the reaction rates (g/kg/h),  $k_{1r}$ ,  $k_{2r}$ , and  $k_{3r}$  are the reaction rate constants (kg/kg/h),  $E_{1B}$  is the bound concentration of cellobiohydrolase and endoglucanase (g/kg),  $E_{2B}$  is the bound concentration of

$\beta$ -glucosidase (g/kg),  $E_{2F}$  is the concentration of  $\beta$ -glucosidase in solution (g/kg),  $R_s$  is the substrate reactivity,  $S$  is the substrate concentration (g/kg),  $G$  is the glucose concentration (g/kg),  $G_2$  is the cellobiose concentration (g/kg),  $X$  is the xylose concentration (g/kg),  $K_{11G}$ ,  $K_{21G}$ , and  $K_{31G}$  are the inhibition constants for glucose (g/kg),  $K_{11G2}$  and  $K_{21G2}$  are the inhibition constants for cellobiose (g/kg),  $K_{11X}$ ,  $K_{21X}$ , and  $K_{31X}$  are the inhibition constants for xylose (g/kg), and  $K_M$  is the substrate (cellobiose) saturation constant. It is assumed that the enzyme and sugar concentrations in free form in the liquid are taken in terms of cellulose as the solid substrate.

Assuming that the normalized initial saccharification rates during secondary hydrolysis of the residual substrates are indicative of substrate reactivity, the following correlation is proposed.

$$R_s = a \frac{S}{S_0} \quad (7.5.11)$$

where  $R_s$  is a substrate reactivity parameter (dimensionless),  $a$  is a constant (dimensionless),  $S$  is the substrate concentration at a given time (g/kg),  $S_0$  is the initial substrate concentration (g/kg), and  $S/S_0$  is the dimensionless substrate concentration at a given time [133].

#### 7.5.3.2.3.2 Enzymatic Saccharification

Unlike acid hydrolysis, enzymatic hydrolysis is highly specific and can produce high yields of relatively pure glucose syrups without generating glucose degradation products. Utility cost of enzymatic hydrolysis is low

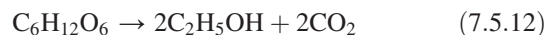
compared to acid or alkaline hydrolysis because enzymatic hydrolysis usually occurs under mild reaction conditions (pH 4.8 and temperature 45–50 °C), does not have a corrosion problem, and holds tremendous promise for the production of fermentation sugars from lignocellulosic biomass. Enzymatic hydrolysis of cellulose consists of the cellulase adsorption onto the surface of the cellulose, the biodegradation of cellulose to fermentable sugars, and desorption of the cellulase. The hydrolytic step is catalyzed by the synergistic action of three major classes of activities encompassed in an enzyme complex referred to as cellulase. These activities consist of (1) endoglucanases, which randomly attacks the cellulose chain to produce polysaccharides of shorter length; (2) exoglucanases, which attaches to the non-reducing ends of these shorter chains and removes cellobiose moieties; and (3)  $\beta$ -glucosidases, which hydrolyzes cellobiose and other oligosaccharides to glucose. Strictly speaking,  $\beta$ -glucosidases is not a cellulase. However, it has a very important role in hydrolysis, as cellobiose is an end-product inhibitor of many cellulases [134]. On the other hand,  $\beta$ -glucosidase is inhibited by glucose [135]. As enzymes are inhibited by the end products, the build-up of any of these products negatively affects cellulose hydrolysis. In addition to the enzymes described earlier, oxidative enzymes are also involved in cellulose degradation. The oxidases oxidize cellobiose and higher cellobioses into their corresponding-ionic acids using molecular oxygen [136].

Both bacteria and fungi can produce cellulases for the hydrolysis of lignocellulosic materials. These microorganisms can be aerobic or anaerobic, mesophilic or thermophilic. Bacteria belonging to *Clostridium*, *Cellulomonas*, *Bacillus*, *Thermomonospora*, *Ruminococcus*, *Bacteriodes*, *Erwinia*, *Acetovibrio*, *Microbispora*, and *Streptomyces* can produce cellulases [137]. *Cellulomonas fimi* and *Thermomonospora fusca* have been extensively studied for cellulase production. Although many cellulolytic bacteria, particularly the cellulolytic anaerobes such as *Clostridium thermocellum* and *Bacteriodes cellulosolvens* produce cellulases with high specificity, they do not produce high enzyme titres. Because the anaerobes have a very low growth rate and require anaerobic growth conditions, most research for commercial cellulase production has focused on fungi [138]. Fungi that have been reported to produce cellulases include *Sclerotium rolfsii*, *P. chrysosporium*, and species of *Trichoderma*, *Aspergillus*, *Schizophyllum*, and *Penicillium* [96, 138, 139]. The cellulase enzymes from *Trichoderma reesei*, formerly known as *Trichoderma viride*, are most extensively studied. This cellulase system was found to contain at least three endoglucanases (EGI, II, and III), two exoglucanases (CBHI, II), and one glucosidase. All these enzymes are glycoproteins with approximately 15% sugar components (based on mass). On the basis of the total secreted protein, the distribution of the three main proteins is 60% exoglucanases, about 5–10%

endoglucanases, and about 1% glucosidase [140]. For the enzymatic conversion of cellulose to glucose on a technical scale, it is necessary to have all cellulolytic components at their optimal level. As the  $\beta$ -glucosidase activity in *T. Reesei* cellulase preparation is below its optimum level [141], it is important to find a way of supplying additional  $\beta$ -glucosidase to such reactions. Therefore, many attempts have therefore been made to find alternative sources of  $\beta$ -glucosidase and also to maximize its production. The black *Aspergilli* (*A. niger* and *A. phoenicia*) were found to be superior producers of  $\beta$ -glucosidases, and a method for production of these enzymes was developed [134]. When *Trichoderma* cellulase preparations were supplemented with  $\beta$ -glucosidases from *Aspergillus* during practical saccharification, glucose was the major product, and the rate of saccharification was significantly increased [142]. Use of a cellulase mixture from different microorganisms or a mixture of cellulases and other enzymes in the hydrolysis of cellulosic materials has been extensively studied [143–145]. The addition of  $\beta$ -glucosidases into the *T. reesei* cellulases system achieved better saccharification than the system without  $\beta$ -glucosidases. A mixture of hemicellulases or pectinases with cellulases exhibited a significant increase in the extent of cellulose conversion [146, 147]. A cellulose conversion yield of 90% was achieved in the enzymatic saccharification of 8% alkali-treated sugarcane bagasse when a mixture of cellulases (dose, 1.0 FPU/g substrate) from *A. ustus* and *T. viride* was used. A nearly complete saccharification of steam-explosion pretreated Eucalyptus viminalis chips (substrate concentration of 6% and enzyme loading of 10 FPU/g cellulose) was obtained using a cellulase mixture of commercial Celluclast and Novozym preparations [148]. Table 7.5.5 summarizes the most recently published results on the enzymatic saccharification and yeast fermentation of lignocellulosic materials.

#### 7.5.3.2.4 Fermentation Strategy

Many organisms grow without using the electron transport chain. The generation of energy without the electron transport chain is called fermentation. This definition is the exact and original meaning of the term fermentation, although currently it is often used in a broader context. The chemical equation below summarizes the fermentation of glucose. One glucose molecule is converted into two ethanol molecules and two carbon dioxide molecules:



As Fig. 7.5.6 shown, this reaction is accompanied by the size difference of two molecules of  $\text{NAD}^+$  to  $\text{NADH}$  and a net of two ADP molecules converted to two ATP plus the two water molecules. Pyruvate is then converted to acetaldehyde and carbon dioxide by an enzyme called pyruvate decarboxylase, which requires thiamine diphosphate as a cofactor. The acetaldehyde is subsequently

**TABLE 7.5.5 Hydrolysis and Fermentation Conditions for Ethanol Production from Different Cereal Materials**

No.	Feedstocks	Pretreatment and saccharification	Microorganisms	Fermentation conditions	Ref.
1	Barley straw	NaOH pretreated, enzyme hydrolysis	<i>Kluyveromyces marxianus</i> IMB3	2, 4, and 6% w/v substrate, 45°C, SHF	[149]
2	Barley straw	H <sub>2</sub> SO <sub>4</sub> catalyzed steam explosion pretreated, enzyme hydrolysis	<i>S. cerevisiae</i>	5, 7.5, and 10% w/w substrate, 35°C, pH 5, SSF	[61]
3	Wheat straw	Alkaline H <sub>2</sub> O <sub>2</sub> pretreated, enzyme hydrolysis	Recombinant <i>E. coli</i> FBR5	37°C, pH 6.5, SHF; 37°C, pH 6.0, SSF	[150]
4	Wheat straw	0.75% v/v dilute H <sub>2</sub> SO <sub>4</sub> pretreated, enzyme hydrolysis	Recombinant <i>E. coli</i> FBR5	35°C, pH 6.5, SHF; 35°C, pH 6.0, SSF	[91]
5	Wheat straw	Lime pretreated, enzyme hydrolysis	Recombinant <i>E. coli</i> FBR5	35°C, pH 6.5, SHF; 35°C, pH 6.0, SSF	[151]
6	Wheat straw	Microwave pretreated, enzyme hydrolysis	Recombinant <i>E. coli</i> FBR5	35°C, pH 6.5, 40 h SHF; 35°C, pH 6.0, 69 h, SSF	[152]
7	Wheat straw	Steam explosion pretreated, enzyme hydrolysis	<i>Kluyveromyces marxianus</i> CECT 10875	42°C, pH 5.5, 72 h, SSF	[153]
8	Wheat straw	H <sub>2</sub> SO <sub>4</sub> catalyzed steam explosion pretreated, enzyme hydrolysis	Recombinant <i>S. cerevisiae</i> USM21 and TMB3400	30, 34, and 37°C, pH 5.0, 96 h, SSF	[154]
9	Wheat straw	0.2% H <sub>2</sub> SO <sub>4</sub> impregnated steam explosion pretreated, enzyme hydrolysis	<i>S. cerevisiae</i>	35°C, pH 5.0, 72 h, SSF	[71]
10	Rice straw	Microwave/acid/alkali/H <sub>2</sub> O <sub>2</sub> pretreated, enzyme hydrolysis	<i>S. cerevisiae</i>	10 w/v substrate, 40°C, pH 5.3, 72 h, SSF	[155]
11	Rice straw	1–3% H <sub>2</sub> SO <sub>4</sub> w/w and steam pretreated, enzyme hydrolysis	<i>P. stipitis</i> BCRC21777	30°C, pH 5.0, 96 h, SSF	[156]
12	rice straw	H <sub>2</sub> SO <sub>4</sub> catalyzed steam pretreated, enzyme hydrolysis	<i>Mucor indicus</i> , <i>Rhizopus oryzae</i> , and <i>S. cerevisiae</i>	30°C, 30 h, SHF	[157]
13	Rice hulls	Alkaline H <sub>2</sub> O <sub>2</sub> pretreated, enzyme hydrolysis	Recombinant <i>E. coli</i> FBR5	35°C, pH 6.5, 24 h SHF; 35°C, pH 6.0, 48 h, SSF	[158]
14	Rice hulls	Lime pretreated, enzyme hydrolysis	Recombinant <i>E. coli</i> FBR5	35°C, pH 6.5, 19 h SHF; 35°C, pH 6.0, 53 h, SSF	[159]
15	Rice hulls	0.4 M H <sub>2</sub> SO <sub>4</sub> hydrolysis	Recombinant <i>E. coli</i> KO11	35°C, SHF	[160]
16	Corn stover	Ammonia pretreated, enzyme hydrolysis	Recombinant <i>E. coli</i> KO11	SSCF	[161]
17	Corn stover	SO <sub>2</sub> catalyzed steam explosion pretreated, enzyme hydrolysis	Recombinant <i>S. cerevisiae</i> TMB3400	35°C, pH 5.0, 96 h, SSCF	[162]
18	Corn stover	SO <sub>2</sub> catalyzed steam explosion pretreated, enzyme hydrolysis	Baker's yeast	30°C, pH 5.5, 144 h, SSF	[163]
19	Corn stover	Alkaline pretreated, enzyme hydrolysis	Recombinant <i>S. cerevisiae</i> ZU 10	33°C, 72 h, SSF	[164]
20	Corn stover	SO <sub>2</sub> catalyzed steam explosion pretreated, enzyme hydrolysis	<i>S. cerevisiae</i>	30°C, pH 5.0, 72 h, SSF	[165]
21	Corn stover	SO <sub>2</sub> catalyzed steam explosion pretreated, enzyme hydrolysis	<i>S. cerevisiae</i>	45°C, pH 5.0, 120 h SHF; 30°C, pH 5.0, 120 h, SSF	[166]
22	Sugarcane bagasse	Alkaline pre pretreated, peracetic acid pretreated, enzyme hydrolysis	Recombinant <i>Z. mobilis</i>	SSCF	[167]
23	Sugarcane bagasse	1.25% w/w H <sub>2</sub> SO <sub>4</sub> hydrolysis	<i>P. tannophilus</i> DW06	30°C, pH 5.5, 30 h, SHF	[168]
24	Sorghum bagasse and wheat straw	Steam explosion pretreated, enzyme hydrolysis	<i>Kluyveromyces marxianus</i> CECT 10875	42°C, 10% w/v substrate, 72–82 h, SSF	[72]



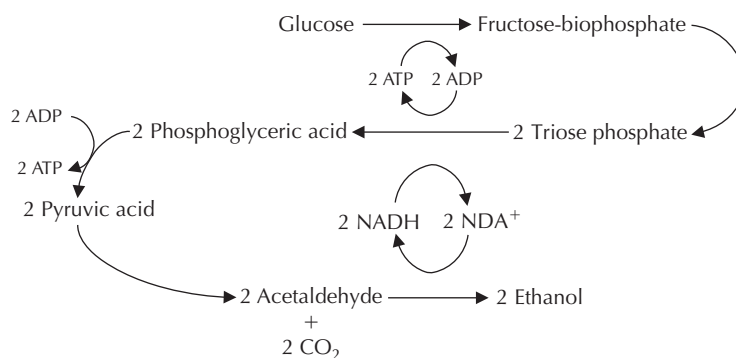


FIGURE 7.5.6 Metabolic pathway from glucose to ethanol by *Saccharomyces cerevisiae*.

reduced to ethanol by the NADH from the previous glycolysis, then NADH is returned to  $\text{NAD}^+$ .

Fermentable sugars, especially glucose, can be converted to other valuable products such as fructose, ethanol, numerous organic acids, and many other products by the enzymatic hydrolysis and biochemical conversion of cellulosic substrates. Production of ethanol from lignocellulosic biomass is a very different system from the method used to extract it from corn because the carbohydrates are much more difficult to be solubilized than the starch in grain. The traditional yeast fermentations cannot be ideally suited to the unique fermentation requirements of cellulose hydrolysates. The basic problems are (1) the current hydrolysis technologies generally produce dilute glucose syrups, (2) the large pentosan fraction is not fermented by traditional brewing yeasts, and (3) the hydrolysates may contain inhibitory compounds generated by the pretreatment process [141]. Research has been progressed on resolving these problems and it appears that a number of novel fermentation techniques will be developed that maximize ethanol production from all fermentable sugars.

#### 7.5.3.2.4.1 Separate Hydrolysis and Fermentation

In the separate hydrolysis and fermentation (SHF) process, the cellulase production, cellulose hydrolysis, and glucose fermentation are carried out in distinct steps. After the feedstock is pretreated to render it susceptible to hydrolysis, a small portion (about 5–10%) is used to produce cellulase and seed cultivation. The cellulase enzyme is then added to the bulk of the pretreated material to hydrolyze the cellulose to glucose. Next, the glucose is sent to one or more reactors to be fermented into ethanol. Finally, the ethanol is recovered from the broth by a distillation process. The primary advantage of this configuration is that the enzyme production, cellulose hydrolysis, and sugar fermentation step can be treated separately, minimizing the interactions between these steps. Moreover, it is possible to carry out the cellulose hydrolysis and fermentation at its own optimum conditions. The main problem with this process is that the cellulase enzyme system is significantly inhibited by the presence of sugars. As a result, the enzymatic hydrolysis

must be conducted in a dilute aqueous solution to achieve a higher sugar yield. Consequently, the process requires large-scale equipment and evaporation of more water in concentrating the sugar solution.

#### 7.5.3.2.4.2 Simultaneous Saccharification and Fermentation

The simultaneous saccharification and fermentation (SSF) process was originally developed at Gulf Oil Chemicals (now Chevron) and the University of Arkansas [169]. In the SSF process, the enzymatic hydrolysis of cellulose into glucose and subsequent glucose fermentation to ethanol is carried out in a single vessel. SSF has the following advantages: (1) increase of hydrolysis rate by conversion of sugars that inhibit the cellulase activity because the end-product inhibition caused by glucose and cellobiose is eliminated; (2) lower enzyme requirement; (3) higher product yields; (4) lower requirements for sterile conditions because glucose is removed immediately and ethanol is produced; (5) shorter process time; (6) less reactor volume because a single reactor is used. Thus, the overall process is carried out at faster rates, higher yields, and greater ethanol concentrations than is possible for the SHF. The presence of ethanol in the fermentation/hydrolysis broth and the low sugar contents reduce the probability of microbial contamination. A significant cost reduction has been achieved through this approach. The cost of ethanol production from biomass was decreased from \$3.6/gal in 1980 to \$1.35/gal in 1988–1990 [52]. The major disadvantage of this process is that the different optimum temperatures for cellulases (45–50 °C) and for ethanol fermenting organisms (20–35 °C) are required. To preserve the viability of the yeast, the SSF process is typically operated under 30–38 °C, at which the cellulase is far below its peak operational level. The optimum temperature for SSF by using *T. reesei* cellulase and *S. cerevisiae* was reported to be around 38 °C, which is a balance between the optimal temperatures for hydrolysis and fermentation. Several thermotolerant bacteria and yeasts, e.g. *Candida acidothermophilum* and *Kluyveromyces marxianus* have been proposed for use in SSF to raise the temperature close to the optimal temperature of



hydrolysis [72, 170–173]. Besides, ethanol may also exhibit inhibition to the cellulase activity in the SSF process. Wu and Lee [174] found that at 38 °C, cellulase lost 9, 36, and 64% of its original activity at ethanol concentrations of 9, 35, and 60 g/L, respectively, during SSF process. However, the ethanol inhibition of cellulase is not very concerned, since practically it is not possible to work with very high substrate concentration in SSF because of the limit of mechanical mixing and insufficient mass transfer. Despite the aforementioned problems, SSF is the preferred method in many laboratory studies and pilot scale studies for ethanol production (Table 7.5.5).

In 1998, a concept of nonisothermal simultaneous saccharification and fermentation process (NSSF) was suggested to overcome the different optimum temperature of enzyme and yeast [175]. In this process, saccharification and fermentation occur simultaneously but in two separate reactors at different temperatures. The lignocellulose is retained inside a hydrolysis reactor and hydrolyzed at the optimum temperature for the enzymatic reactions (e.g. 50 °C). The effluent from the reactor is recirculated through a fermentor, which runs at its optimum temperature (e.g. 30 °C). The cellulase activity is increased 2–3 times when the hydrolysis temperature is raised from 30 to 50 °C. The NSSF process has improved the kinetic enzymatic reaction compared to SSF, resulting in reduction of the overall enzyme requirement by 30–40%. The overall time needed in NSSF was much lower than that of SSF. The terminal yield, which was obtained with SSF in 4 days, was obtained in 40 h with the NSSF [175]. Varga et al. [176] and Kadar et al. [177] introduced another form of NSSF for the production of ethanol from corn stover and industrial waste, respectively. They proposed prehydrolysis at 50 °C, the optimal temperature of enzymes, and then the media was inoculated with *S. cerevisiae* or *Kluyveromyces marxianus* and incubated at 30 °C.

Contrary to sucrose- and starch-based ethanol production, lignocellulose-based ethanol production is a mixed-sugar fermentation in the presence of inhibiting compounds—low molecular weight organic acids, furan derivatives, phenolics, and inorganic compounds—released and formed during pretreatment and/or hydrolysis of the raw material. The sugars derived from biomass is a mixture of hexoses (primarily glucose) and pentoses (primarily xylose), with significantly less amount of arabinose, galactose, and mannose. Hence, another mode of operation called simultaneous saccharification and co-fermentation (SSCF) was induced to allow the hemicelluloses sugars to be converted to ethanol together with SSF of the cellulose. In SSCF process, it is suggested to ferment both hexoses and pentoses in a single reactor with a single microorganism. However, most wild-type strains of *S. cerevisiae*, which are most commercially applied in the six-carbon sugar fermentation, do not metabolize xylose. In nature, xylose-fermenting yeast, notably *Pichia stipitis*,

ferment xylose to ethanol with reasonable yield and productivity; however, these yeast strains are inhibited by compounds generated during pretreatment and hydrolysis of the lignocellulose material [178]. In recent years, metabolic engineering concepts have been used for the production of fuel ethanol [179]. In the first approach, xylose-metabolizing genes have been engineered into wild-type ethanologens such as the yeast *Saccharomyces cerevisiae* and the bacteria *Escherichia coli*, *Klebsiella oxytoca*, and *Zymomonas mobilis* [179–181]. Recombinant strains of *S. cerevisiae* with the ability to coferment glucose and xylose have been constructed by adding *Pichia stipitis* genes (XYL1, XYL2) for an NADPH-dependent xylose reductase and a NAD<sup>+</sup>-dependent xylitol dehydrogenase, and by enhancing expression of the endogenous xylulokinase. Thus, in three steps, xylose is converted to xylulose-5-phosphate, which is a central metabolite of the endogenous pentose phosphate pathway [182]. The genetically engineered *Escherichia coli* KO11, *Klebsiella oxytoca* P2, and *Zymomonas mobilis* AX101 are considered for commercial scale-up [183]. Recombinant strains of *Saccharomyces cerevisiae* have also been genetically engineered to carry out SSF to produce extracellular endoglucanase and  $\beta$ -glucosidase that are able to ferment cellulose and hemicelluloses to C<sub>6</sub> and C<sub>5</sub> sugars and subsequent fermentation to ethanol [183–186]. The recombinant yeast strain *S. cerevisiae* MT8-1 was found to ferment xylose and cello-oligosaccharides by integrating genes from *Pichia stipitis* that express xylose reductase and xylitol dehydrogenase, and xylulokinase from *Saccharomyces cerevisiae* and a gene for displaying  $\beta$ -glucosidase from *Aspergillus aculeatus* on the cell surface [187].

## 7.5.4 SUMMARY

Ultimately, biomass conversion processes are attractive because they are in practice today, and extension to future scenarios is easy to envision. In recent years, much valuable work has been performed on different aspects of ethanol production from lignocelluloses based on enzymatic hydrolysis, and great achievements have been attained. Although the cost of feedstock and cellulolytic enzymes are the two important parameters and are the main obstacles for industrial bioethanol production, process integration and optimization, together with the research on the genetically engineered enzyme and yeast, will improve the energy consumption of the process. Scientists' efforts will help to improve the economy of the process by reducing the prices of cellulase, as well as optimizing the enzymatic hydrolysis process. These strivings in basic and applied sciences are believed to be essential to lead the world toward clean and reliable sources of energy.

## REFERENCES

- [1] L.R. Lynd, H. Jin, J.G. Michels, C.E. Wyman, B. Dale, Bioenergy: background, potential, and policy. A policy briefing prepared for the Center for Strategic and International Studies, Washington, DC, 2003, <http://www.agriculture.senate.gov/Hearings/hearings.cfm?hearingid=1161&witnessId=3320>.
- [2] C.J. Campbell, Peak oil: an outlook on crude oil depletion, 2002, <http://www.mbendi.com/indy/oil/gp0070.htm>.
- [3] K.S. Deffeyes, Hubbert's Peak: The Impending Oil Shortage, Princeton University Press, Princeton, NJ, 2001.
- [4] M. Galbe, G. Zacchi, A review of the production of ethanol from softwood, *Appl. Microbiol. Biotechnol.* 59 (2002) 618–628.
- [5] Biofuels in the European Union: A Vision for 2030 and Beyond, 2006, <http://www.biomatnet.org/publications/1919rep.pdf>.
- [6] S. Herrera, Bonkers about biofuels, *Nat. Biotechnol.* 24 (2006) 755–760.
- [7] Y. Lin, S. Tanaka, Ethanol fermentation from biomass resources: current state and prospects, *Appl. Microbiol. Biotechnol.* 69 (2006) 627–642.
- [8] B. Hahn Hagerdal, M. Galbe, M.F. Gorwa Grauslund, G. Liden, G. Zacchi, Bio ethanol the fuel of tomorrow from the residues of today, *Trends Biotechnol.* 24 (2006) 549–556.
- [9] J.R. Mielenz, Ethanol production from biomass: technology and commercialization status, *Curr. Opin. Microbiol.* 4, 2001, 324–329.
- [10] C.E. Wyman, Ethanol production from lignocellulosic biomass: overview, in: C.E. Wyman (Ed.), *Handbook on Bioethanol: Production and Utilization*, Taylor & Francis Group, Washington, DC, 1996, pp. 11–18.
- [11] L.R. Lynd, J.H. Cushman, R.J. Nichols, C.E. Wyman, Ethanol from cellulosic biomass, *Science* 251 (1991) 1318–1323.
- [12] B.K. Bailey, Performance of ethanol as a transportation fuel, in: C.E. Wyman (Ed.), *Handbook on Bioethanol: Production and Utilization*, Taylor & Francis, Washington, DC, 1996, pp. 37–60.
- [13] C.E. Wyman, N.D. Hinman, Ethanol: fundamentals of production from renewable feedstocks and use as a transportation fuel, *Appl. Microbiol. Biotechnol.* 24–25 (1990) 735–753.
- [14] Market Research Analyst: World's Ethanol Production Forecast 2008–2012, 2008, <http://www.marketresearchanalyst.com>.
- [15] T. Francl, Ethanol production sets another record in 2001, American Farm Bureau Federation Staff Analyses of Today's Ag Issues, 2002, [http://www.fb.com/issues/analysis/Ethanol\\_Production.html](http://www.fb.com/issues/analysis/Ethanol_Production.html).
- [16] K.H. Kim, M.P. Tucker, Q.A. Nguyen, Effects of pressing lignocellulosic biomass on sugar yield in two stage dilute acid hydrolysis process, *Biotechnol. Progr.* 18 (2002) 489–494.
- [17] K.L. Kadam, J.D. McMillan, Availability of corn stover as a sustainable feedstock for bioethanol production, *Bioresour. Technol.* 88 (2003) 17–25.
- [18] S. Kim, B.E. Dale, Global potential bioethanol production from wasted crops and crop residues, *Biomass Bioenergy* 26 (2004) 361–375.
- [19] M.E. Himmel, Shi. Y. Ding, D.K. Johnson, W.S. Adney, M.R. Nimlos, J.W. Brady, T.D. Foust, Biomass recalcitrance: engineering plants and enzymes for biofuels production, *Science* 315 (2007) 804–807.
- [20] [http://www.biology.unm.edu/ccouncil/Biology\\_203/Summaries/FloweringPlants.htm](http://www.biology.unm.edu/ccouncil/Biology_203/Summaries/FloweringPlants.htm).
- [21] K. Iiyama, T.B.T. Lam, B.A. Stone, Covalent cross links in the cell wall, *Plant Physiol.* 104 (1994) 315–320.
- [22] M.E. Himmel, M.F. Ruth, C.E. Wyman, Cellulase for commodity products from cellulosic biomass, *Curr. Opin. Biotechnol.* 10 (1999) 358–364.
- [23] C.E. Wyman, B.E. Dale, R.T. Elander, M. Holtzapfel, M.R. Ladisch, Y.Y. Lee, Coordinated development of leading biomass pretreatment technologies, *Bioresour. Technol.* 96 (2005) 1959–1966.
- [24] Y. Nishiyama, P. Langan, H. Chanzy, Crystal structure and hydrogen bonding system in cellulose I<sub>β</sub> from synchrotron X ray and neutron fiber diffraction, *J. Am. Chem. Soc.* 124 (2002) 9074–9082.
- [25] M.J. Taherzadeh, K. Karimi, Acid based hydrolysis processes for ethanol from lignocellulose materials: a review, *Bioresour. Technol.* 2 (2007) 472–499.
- [26] E.E. Harris, E. Beglinger, Madison wood sugar process, *Ind. Eng. Chem.* 38 (1946) 890–895.
- [27] F.A. Keller Jr., Integrated bioprocess development for bioethanol production, in: C.E. Wyman (Ed.), *Handbook on Bioethanol: Production and Utilization*, Taylor & Francis, Washington, DC, 1996, pp. 351–380.
- [28] J.F. Saeman, Kinetics of wood saccharification hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature, *Ind. Eng. Chem.* 37 (1945) 43–52.
- [29] A. Rodriguez Chonga, J.A. Ramirez, G. Garrote, M. Vazquez, Hydrolysis of sugar cane bagasse using nitric acid: a kinetic assessment, *J. Food Eng.* 61 (2004) 143–152.
- [30] S. Gameza, J.J. Gonzalez Cabriales, J.A. Ramirez, G. Garrote, M. Vazquez, Study of the hydrolysis of sugar cane bagasse using phosphoric acid, *J. Food Eng.* 74 (2006) 78–88.
- [31] E.E. Harris, Wood saccharification, *Adv. Carbohydr. Chem. Biochem.* 4 (1949) 153–188.
- [32] P.C. Badger, Ethanol from cellulose: a general review, in: J. Janick, A. Whipkey (Eds.), *Trends in New Crops and New Uses*, ASHS Press, Alexandria, 2002, pp. 17–21.
- [33] W.A. Farone, J.E. Cuzens, Method of producing sugars using strong acid hydrolysis of cellulosic and hemicellulosic materials, USA Patent 5562777, 1996.
- [34] W.A. Farone, J.E. Cuzens, Method of separating acids and sugars resulting from strong acid hydrolysis, USA Patent 5580389, 1996.
- [35] N. Iraj, E. Giti, A. Lila, Isolation of a flocculating *Saccharomyces cerevisiae* and investigation of its performance in the fermentation of beet molasses to ethanol, *Biomass Bioenergy* 23 (2002) 481–486.
- [36] J.L. Jones, K.T. Semrau, Wood hydrolysis for ethanol production previous experience and the economics of selected processes, *Biomass* 5 (1984) 109–135.
- [37] M. von Sivers, G. Zacchi, A techno economical comparison of three processes for the production of ethanol from pine, *Bioresour. Technol.* 51 (1995) 43–52.
- [38] J.W. van Groenestijn, J.H.O. Hazewinkel, R.R. Bakker, Pre treatment of ligno cellulose with biological acid recycling (the Biosulfurol process), *Int. Sugar J.* 110 (2008) 689–692.
- [39] <http://www.arkenol.com>.
- [40] <http://www.madada.com>.
- [41] W.L. Faith, Development of the Scholler process in the United States, *Ind. Eng. Chem.* 37 (1945) 9–11.
- [42] Y. Sun, J.Y. Cheng, Hydrolysis of lignocellulosic materials for ethanol production: a review, *Bioresour. Technol.* 83 (2002) 1–11.
- [43] S. Larsson, E. Palmqvist, B. Hahn Hagerdal, C. Tengborg, K. Stenberg, G. Zacchi, N. O. Nilvebrant, The generation of fermentation inhibitors during dilute acid hydrolysis of soft wood, *Enzyme Microb. Technol.* 24 (1999) 151–159.
- [44] H.B. Klinker, A.B. Thomsen, B.K. Ahring, Inhibition of ethanol producing yeast and bacteria by degradation products produced during pretreatment of biomass, *Appl. Microbiol. Biotechnol.* 66 (2004) 10–26.

- [45] L. Olsson, B. Hahn Hagerdal, Fermentation of lignocellulosic hydrolysates for ethanol production, *Enzyme Microb. Technol.* 18 (1996) 312–331.
- [46] K. Karimi, S. Kheradmandinia, M.J. Taherzadeh, Conversion of rice straw to sugars by dilute acid hydrolysis, *Biomass Bioenergy* 30 (2006) 247–253.
- [47] G. Sanchez, L. Pilcher, C. Roslander, T. Modig, M. Galbe, G. Liden, Dilute acid hydrolysis for fermentation of the Bolivian straw material Paja brava, *Bioresour. Technol.* 93 (2004) 249–256.
- [48] E.A. Bayer, L.J. Shimon, Y. Shoham, R. Lamed, Cellulosomes structure and ultrastructure, *J. Struct. Biol.* 124 (1998) 221–234.
- [49] E.A. Bayer, J.P. Belaich, Y. Shoham, R. Lamed, The cellulosomes: multienzyme machines for degradation of plant cell wall polysaccharides, *Annu. Rev. Microbiol.* 58 (2004) 521–554.
- [50] M.E. Himmel, W.A. Adney, J.O. Baker, R. Elander, J.D. McMillan, R.A. Nieves, J.J. Sheehan, S.R. Thomas, T.B. Vinzant, M. Zhang, Advanced bioethanol production technologies: a perspective, in: B.C. Saha, J. Woodward (Eds.), *Fuels and Chemicals from Biomass*, American Chemical Society Symposium Series 666, Washington, DC, 1997, pp. 2–45.
- [51] J.D. Wright, Ethanol from biomass by enzymatic hydrolysis, *Chem. Eng. Prog.* 84 (1988) 62–79.
- [52] S. Kim, M.T. Holtzapfel, Lime pretreatment and enzymatic hydrolysis of corn stover, *Bioresour. Technol.* 96 (2005) 1994–2006.
- [53] M.M. Wu, K. Chang, D.J. Gregg, A. Boussaid, R.P. Beatson, J.N. Saddler, Optimization of steam explosion to enhance hemicellulose recovery and enzymatic hydrolysis of cellulose in softwoods, *Appl. Biochem. Biotechnol.* 77–79 (1999) 47–54.
- [54] T.A. Clark, K.L. Mackie, Steam explosion of the softwood *Pinus radiata* with sulphur dioxide addition. I. Process optimisation, *J. Wood Chem. Technol.* 7 (1987) 373–403.
- [55] S.M. Shevchenko, R.P. Beatson, J.N. Saddler, The nature of lignin from steam explosion/enzymatic hydrolysis of softwood: structural features and possible uses: scientific note, *Appl. Biochem. Biotechnol.* 77–79 (1999) 867–876.
- [56] G. Michalowicz, B. Toussaint, M.R. Vignon, Ultrastructural changes in poplar cell wall during steam explosion treatment, *Holzforschung* 45 (1991) 175–179.
- [57] J.N. Saddler, L.P. Ramos, C. Breuil, Steam pretreatment of lignocellulosic residues, in: J.N. Saddler (Ed.), *Bioconversion of Forest and Agricultural Plant Residues*, C.A.B. International, Wallingford, 1993, pp. 73–92.
- [58] L.P. Walker, D.B. Wilson, Enzymatic hydrolysis of cellulose: an overview, *Bioresour. Technol.* 36 (1991) 3–14.
- [59] E. Viola, F. Zimbardi, M. Cardinale, G. Cardinale, G. Braccio, E. Gambacorta, Processing cereal straws by steam explosion in a pilot plant to enhance digestibility in ruminants, *Bioresour. Technol.* 99 (2008) 681–689.
- [60] K. Pakkala, M. Kontturi, A. Kallioinen, O. Myllymaki, J. Uusitalo, M. Siika aho, N. von Weymarn, Production of Bioethanol from Barley Straw and Reed Canary Grass: A Raw Material Study, 15th European Biomass Conference & Exhibition, Berlin, Germany, 2007, pp. 154–157.
- [61] M. Linde, M. Galbe, G. Zacchi, Simultaneous saccharification and fermentation of steam pretreated barley straw at low enzyme loadings and low yeast concentration, *Enzyme Microb. Technol.* 40 (2007) 1100–1107.
- [62] E.Y. Vlasenko, H. Ding, J.M. Labavitch, S.P. Shoemaker, Enzymatic hydrolysis of pretreated rice straw, *Bioresour. Technol.* 59 (1997) 109–119.
- [63] M. Moniruzzaman, Saccharification and alcohol fermentation of steam exploded rice straw, *Bioresour. Technol.* 55 (1996) 111–117.
- [64] M. Abbi, R.C. Kuhad, A. Singh, Bioconversion of pentose sugars to ethanol by free and immobilized cells of *Candida shehatae* (NCL 3501): fermentation behaviour, *Process Biochem.* 31 (1996) 555–560.
- [65] M.G. Tabka, I. Herpoel Gimbert, F. Monod, M. Asther, J.C. Sigoillot, Enzymatic saccharification of wheat straw for bioethanol production by a combined cellulase xylanase and feruloyl esterase treatment, *Enzyme Microb. Technol.* 39 (2006) 897–902.
- [66] X.F. Sun, F. Xu, R.C. Sun, P. Fowler, M.S. Baird, Characteristics of degraded cellulose obtained from steam exploded wheat straw, *Carbohydr. Res.* 340 (2005) 97–106.
- [67] D. Montane, X. Farriol, J. Salvado, P. Jollez, E. Chornet, Fractionation of wheat straw by steam explosion pretreatment and alkali delignification. Cellulose pulp and byproducts from hemicellulose and lignin, *J. Wood Chem. Technol.* 18 (1998) 171–191.
- [68] H.Z. Chen, Y.J. Han, J. Xu, Simultaneous saccharification and fermentation of steam exploded wheat straw pretreated with alkaline peroxide, *Process Biochem.* 43 (2008) 1462–1466.
- [69] F. Zimbardi, D. Viggiano, F. Nanna, M. Demichele, D. Cuna, G. Cardinale, Steam explosion of straw in batch and continuous systems, *Appl. Biochem. Biotechnol.* 77–79 (1999) 117–125.
- [70] I. Ballesteros, M.J. Negro, J.M. Oliva, A. Cabanas, P. Manzanares, M. Ballesteros, Ethanol production from steam explosion pretreated wheat straw, *Appl. Biochem. Biotechnol.* 130 (2006) 496–508.
- [71] M. Linde, E.L. Jakobsson, M. Galbe, G. Zacchi, Steam pretreatment of dilute H<sub>2</sub>SO<sub>4</sub> impregnated wheat straw and SSF with low yeast and enzyme loadings for bioethanol production, *Biomass Bioenergy* 32 (2008) 326–332.
- [72] M. Ballesteros, J.M. Oliva, M.J. Negro, P. Manzanares, I. Ballesteros, Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with *Kluyveromyces marxianus* CECT 10875, *Process Biochem.* 39 (2004) 1843–1848.
- [73] W.E. Kaar, C.V. Gutierrez, C.M. Kinoshita, Steam explosion of sugarcane bagasse as a pretreatment for conversion to ethanol, *Biomass Bioenergy* 14 (1998) 277–287.
- [74] M. Laser, D. Schulman, S.G. Allen, J. Lichwa, M.J. Antal, L.R. Lynd, A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol, *Bioresour. Technol.* 81 (2002) 33–44.
- [75] K. Ohgren, M. Galbe, G. Zacchi, Optimization of steam pretreatment of SO<sub>2</sub> impregnated corn stover for fuel ethanol production, *Appl. Biochem. Biotechnol.* 121–124 (2005) 1055–1067.
- [76] M.P. Tucker, K.H. Kim, M.M. Newman, Q.A. Nguyen, Effects of temperature and moisture on dilute acid steam explosion pretreatment of corn stover and cellulase enzyme digestibility, *Appl. Biochem. Biotechnol.* 105–108 (2003) 165–177.
- [77] F. Zimbardi, E. Viola, F. Nanna, E. Larocca, M. Cardinale, D. Barisano, Acid impregnation and steam explosion of corn stover in batch processes, *Ind. Crops Prod.* 26 (2007) 195–206.
- [78] E. Varga, K. Reczey, G. Zacchi, Optimization of steam pretreatment of corn stover to enhance enzymatic digestibility, *Appl. Biochem. Biotechnol.* 113–116 (2004) 509–523.
- [79] S.P.S. Chundawat, B. Venkateshi, B.E. Dale, Effect of particle size based separation of milled corn stover on AFEX pretreatment and enzymatic digestibility, *Biotechnol. Bioeng.* 96 (2007) 219–231.
- [80] T.H. Kim, J.S. Kim, C. Sunwoo, Y.Y. Lee, Pretreatment of corn stover by aqueous ammonia, *Bioresour. Technol.* 90 (2003) 39–47.

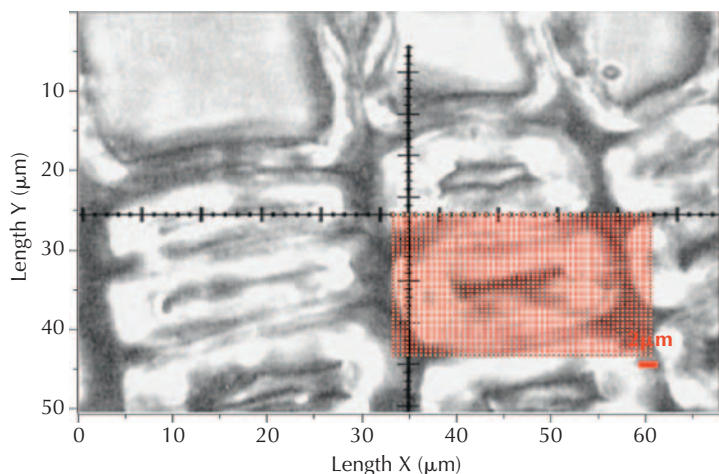
- [81] E. Viola, M. Cardinale, R. Santarcangelo, A. Villone, F. Zimbardi, Ethanol from eel grass via steam explosion and enzymatic hydrolysis, *Biomass Bioenergy* 32 (2008) 613–618.
- [82] R.P. Overend, E. Chornet, J.A. Gascoigne, Fractionation of lignocellulosics by steam aqueous pretreatments [and Discussion], *Philos. Trans. R. Soc. Lond. A* 321 (1987) 523–536.
- [83] H.L. Chum, D.K. Johnson, S.K. Black, R.P. Overend, Pretreatment catalyst effects and the combined severity parameter, *Appl. Biochem. Biotechnol.* 24–25 (1990) 1–14.
- [84] K.L. Mackie, H.H. Brownell, K.L. West, J.N. Saddler, Effect of sulphur dioxide and sulphuric acid on steam explosion of aspen wood, *J. Wood Chem. Technol.* 5 (1985) 405–425.
- [85] A. Esteghlalian, A.G. Hashimoto, J.J. Fenske, M.H. Penner, Modeling and optimization of the dilute sulfuric acid pretreatment of corn stover, poplar and switchgrass, *Bioresour. Technol.* 59 (1997) 129–136.
- [86] J.D. McMillan, Hemicellulose conversion to ethanol, in: C.E. Wyman (Ed.), *Handbook on Bioethanol: Production and Utilization*, Taylor & Francis, Washington, DC, 1996, pp. 287–313.
- [87] Y. Sun, J.J. Cheng, Dilute acid pretreatment of rye straw and bermudagrass for ethanol production, *Bioresour. Technol.* 96 (2005) 1599–1606.
- [88] K. Grohmann, R. Torget, M. Himmel, Optimization of dilute acid pretreatment of biomass, *Biotechnol. Bioeng. Symp.* 15 (1986) 59–80.
- [89] R. Torget, P. Werdene, M. Himmel, K. Grohmann, Dilute acid pretreatment of short rotation woody and herbaceous crops, *Appl. Biochem. Biotechnol.* 24–25 (1990) 115–126.
- [90] X.B. Zhao, L.H. Zhang, D.H. Liu, Comparative study on chemical pretreatment methods for improving enzymatic digestibility of crofton weed stem, *Bioresour. Technol.* 99 (2008) 3729–3736.
- [91] B.C. Saha, L.B. Iten, M.A. Cotta, Y.V. Wu, Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol, *Process Biochem.* 40 (2005) 3693–3700.
- [92] I.C. Roberto, S.I. Mussatto, R.C.L.B. Rodrigues, Dilute acid hydrolysis for optimization of xylose recovery from rice straw in a semi pilot reactor, *Ind. Crops Prod.* 17 (2003) 171–176.
- [93] D. Jalc, F. Nerud, P. Siroka, The effectiveness of biological treatment of wheat straw by white rot fungi, *Folia Microbiol.* 43 (1998) 687–689.
- [94] M.L. Rabinovich, A.V. Bolobova, L.G. Vasil'chenko, Fungal decomposition of natural aromatic structures and xenobiotics: a review, *Appl. Biochem. Microbiol.* 40 (2004) 1–17.
- [95] J.W. Bennet, K.G. Wunch, B.D. Faison, Use of fungi biodegradation, in: C.J. Hurst (Ed.), *Manual of Environmental Microbiology*, ASM Press, Washington, DC, 2002, pp. 960–971.
- [96] L.T. Fan, M.M. Gharpuray, Y. H. Lee, Cellulose Hydrolysis. *Biotechnology Monographs*, vol. 3. Springer, Berlin, 1987.
- [97] H. Jorgensen, T. Eriksson, J. Borjesson, F. Tjerneld, L. Olsson, Purification and characterization of five cellulases and one xylanase from *Penicillium brasilianum* IBT 20888, *Enzyme Microb. Technol.* 32 (2003) 851–861.
- [98] G. Emtiazi, N. Naghavi, A. Bordbar, Biodegradation of lignocellulosic waste by *Aspergillus terreus*, *Biodegradation* 12 (2001) 259–263.
- [99] Y. Hadar, Z. Kerem, B. Gorodecki, Biodegradation of lignocellulosic agricultural wastes by *Pleurotus ostreatus*, *J. Biotechnol.* 30 (1993) 133–139.
- [100] F.A. Keller, J.E. Hamilton, Q.A. Nguyen, Microbial pretreatment of biomass: potential for reducing severity of thermochemical biomass pretreatment, *Appl. Biochem. Biotechnol.* 105–108 (2003) 27–41.
- [101] R. Ragunathan, K. Swaminathan, Bioconversion of lignocellulosic agro wastes by fungus, *Pleurotus* spp., *Biol. Mem.* 30 (2004) 1–6.
- [102] E. Locci, S. Laconi, R. Pompei, P. Scano, A. Lai, F.C. Marincola, Wheat bran biodegradation by *Pleurotus ostreatus*: a solid state carbon 13 NMR study, *Bioresour. Technol.* 99 (2008) 4279–4284.
- [103] S.M. Hossain, M. Das, S.H. Ibrahim, Studies on biopulping of bagasse using phanerochaete chrysosporium, *J. Ind. Pollut. Control* 19 (2003) 2255–2269.
- [104] A.T. Marquez Araque, G.D.M. Martinez, S.S.G. Munoz, S.E.B. Dios, O.L. Corral, Fibrolytic activity of enzymes produced by *Trametes* sp. EUM1, *Pleurotus ostreatus* IE8 and *Aspergillus niger* AD 96.4 in solid fermentation, *Interciencia* 32 (2007) 780–785.
- [105] N. Moredo, M. Lorenzo, A. Dominguez, D. Moldes, C. Cameselle, A. Sanroman, Enhanced ligninolytic enzyme production and degrading capability of *Panerochaete chrysosporium* and *Trametes versicolor*, *World J. Microbiol. Biotechnol.* 19 (2003) 665–669.
- [106] I. Delfin Alcala, C. Duran De Bazua, Cellulosic urban residues biodegraded by *Pleurotus*, *Rev. Int. Contam. Ambient* 19 (2003) 37–45.
- [107] F.H. Shah, A. Majid, R. Zia ur, Symbiotic biodegradation of crop residues. I. Biodegradation of wheat and rice straw, *Pak. J. Sci. Ind. Res.* 26 (1982) 31–34.
- [108] A.I. Hatakka, Pretreatment of wheat straw by white rot fungi for enzymic saccharification of cellulose, *Appl. Microbiol. Biotechnol.* 18 (1983) 350–357.
- [109] B.A. Summerell, L.W. Burgess, Decomposition and chemical composition of cereal straw, *Soil Biol. Biochem.* 21 (1989) 551–559.
- [110] K. Karunanadaa, S.L. Fales, G.A. Varga, D.J. Royse, Chemical composition and biodegradability of crop residues colonized by white rot fungi, *J. Sci. Food Agric.* 60 (1992) 105–112.
- [111] D.E. Akin, L.L. Rigsby, A. Sethuraman, W.H. Morrison, G.R. Gamble, K.E. Eriksson, Alterations in structure, chemistry, and biodegradability of grass lignocellulose treated with the white rot fungi *Ceriporiopsis subvermispora* and *Cyathus stercoreus*, *Appl. Environ. Microbiol.* 61 (1995) 1591–1598.
- [112] T.A. Clark, K.L. Mackie, Fermentation inhibitors in wood hydrolysates derived from the softwood *Pinus radiata*, *J. Chem. Technol. Biotechnol. B: Biotechnol.* 34 (1984) 101–110.
- [113] M.J. Taherzadeh, C. Niklasson, G. Liden, Conversion of dilute acid hydrolyzates of spruce and birch to ethanol by fed batch fermentation, *Bioresour. Technol.* 69 (1999) 59–66.
- [114] M.J. Taherzadeh, C. Niklasson, G. Liden, Acetic acid friend or foe in anaerobic batch conversion of glucose to ethanol by *Saccharomyces cerevisiae*, *Chem. Eng. Sci.* 52 (1997) 2653–2659.
- [115] Q. Xiang, Y.Y. Lee, R.W. Torget, Kinetics of glucose decomposition during dilute acid hydrolysis of lignocellulosic biomass, *Appl. Biochem. Biotechnol.* 113–116 (2004) 1127–1138.
- [116] E. Palmqvist, B. Hahn Hagerdal, Fermentation of lignocellulosic hydrolysates. II. Inhibitors and mechanisms of inhibition, *Bioresour. Technol.* 74 (2000) 25–33.
- [117] N.K. Nishikawa, R. Sutcliffe, J.N. Saddler, The influence of lignin degradation products on xylose fermentation by *Klebsiella pneumoniae*, *Appl. Microbiol. Biotechnol.* 27 (1988) 549–552.
- [118] S. Larsson, A. Reimann, N. O. Nilvebrant, L.J. Jonsson, Comparison of different methods for the detoxification of lignocellulose hydrolyzates of spruce, *Appl. Biochem. Biotechnol.* 77 (1999) 91–103.
- [119] A. Petersson, J.R. Almeida, T. Modig, K. Karhumaa, B. Hahn Hagerdal, M.F. Gorwa Grauslund, G. Liden, A 5-hydroxymethyl



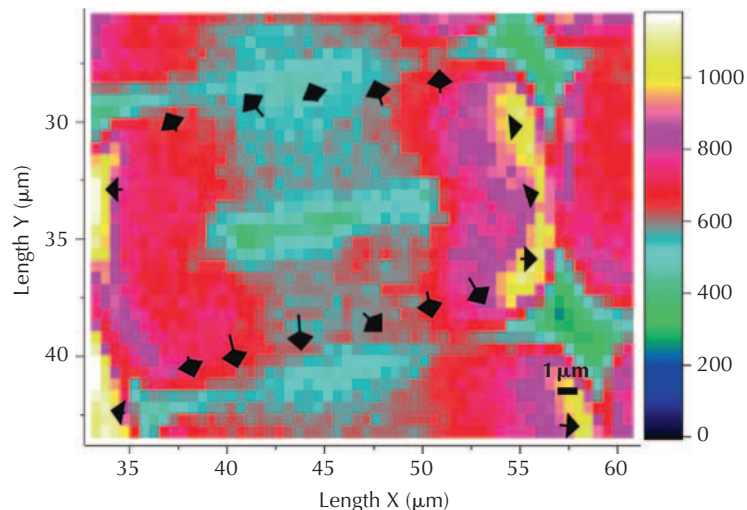
- furfural reducing enzyme encoded by the *Saccharomyces cerevisiae* ADH6 gene conveys HMF tolerance, *Yeast* 23 (2006) 455–464.
- [120] H.Q. Li, H.Z. Chen, Detoxification of steam exploded corn straw produced by an industrial scale reactor, *Process Biochem.* 43 (2008) 1447–1451.
- [121] S.I. Mussatto, I.C. Roberto, Hydrolysate detoxification with activated charcoal for xylitol production by *Candida guilliermondii*, *Biotechnol. Lett.* 23 (2001) 1681–1684.
- [122] E. Sepulveda Huerta, S.J. Tellez Luis, V. Bocanegra Garcia, J.A. Ramirez, M. Vazquez, Production of detoxified sorghum straw hydrolysates for fermentative purposes, *J. Sci. Food Agric.* 86 (2006) 2579–2586.
- [123] S. Amartei, T. Jeffries, An improvement in *Pichia stipitis* fermentation of acid hydrolysed hemicellulose achieved by over liming (calcium hydroxide treatment) and strain adaptation, *World J. Microbiol. Biotechnol.* 12 (1996) 281–283.
- [124] K.K. Cheng, B.Y. Cai, J.A. Zhang, H.Z. Ling, Y.J. Zhou, J.P. Ge, J.M. Xu, Sugarcane bagasse hemicellulose hydrolysate for ethanol production by acid recovery process, *Biochem. Eng. J.* 38 (2008) 105–109.
- [125] A.K. Chandel, R.K. Kapoor, A. Singh, R.C. Kuhad, Detoxification of sugarcane bagasse hydrolysate improves ethanol production by *Candida shehatae* NCIM 3501, *Bioresour. Technol.* 98 (2007) 1947–1950.
- [126] J.N. Nigam, Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*, *J. Biotechnol.* 87 (2001) 17–27.
- [127] L.A. Alves, M.G.A. Felipe, E. Almeida, J.B. Silva, S.S. Silva, A.M.R. Prata, Pretreatment of sugarcane bagasse hemicellulose hydrolysate for xylitol production by *Candida guilliermondii*, *Appl. Biochem. Biotechnol.* 70–72 (1998) 89–98.
- [128] C.S. Gong, C.S. Chen, L.F. Chen, Pretreatment of sugar cane bagasse hemicellulose hydrolyzate for ethanol production by yeast, *Appl. Biochem. Biotechnol.* 39–40 (1993) 83–88.
- [129] N.O. Nilvebrant, A. Reimann, S. Larsson, L.J. Jonsson, Detoxification of lignocellulose hydrolysates with ion exchange resins, *Appl. Biochem. Biotechnol.* 91–93 (2001) 35–49.
- [130] M. Cantarelli, L. Cantarella, A.I. Gallifuoco, A. Spera, F. Alfani, Comparison of different detoxification methods for steam exploded poplar wood as a substrate for the bioproduction of ethanol in SHF and SSF, *Process Biochem.* 39 (2004) 1533–1542.
- [131] L.J. Jonsson, E. Palmqvist, N. O. Nilvebrant, B. Hahn Hagerdal, Detoxification of wood hydrolysates with laccase and peroxidase from the white rot fungus *Trametes versicolor*, *Appl. Microbiol. Biotechnol.* 49 (1998) 691–697.
- [132] K.L. Kadam, E.C. Rydholm, J.D. McMillan, Development and validation of a kinetic model for enzymatic saccharification of lignocellulosic biomass, *Biotechnol. Progr.* 20 (2004) 698–705.
- [133] S. Kumar, S.P. Singh, I.M. Mishra, D.K. Adhikari, Recent advances in production of bioethanol from lignocellulosic biomass, *Chem. Eng. Technol.* 32 (2009) 517–526.
- [134] D. Sternberg, P. Vuayakumar, E.T. Reese,  $\beta$  Glucosidase: microbial production and effect on enzymatic hydrolysis of cellulose, *Can. J. Microbiol.* 23 (1977) 139–147.
- [135] M. Holtzapfel, M. Cognata, Y. Shu, C. Hendrickson, Inhibition of *Trichoderma reesei* cellulase by sugars and solvents, *Biotechnol. Bioeng.* 36 (1990) 275–287.
- [136] A.R. Ayers, S.B. Ayers, K.E. Eriksson, Cellobiose oxidase, purification and partial characterization of a hemoprotein from *Sporotrichum pulverulentum*, *Eur. J. Biochem.* 90 (1978) 171–181.
- [137] V.S. Bisaria, Bioprocessing of agro residues to glucose and chemicals, in: A.M. Martin (Ed.), *Bioconversion of Caste Materials to Industrial Products*, Elsevier, London, 1991, pp. 210–213.
- [138] S.J.B. Duff, W.D. Murray, Bioconversion of forest products industry waste cellulose to fuel ethanol: a review, *Bioresour. Technol.* 55 (1996) 1–33.
- [139] D. Sternberg, Production of cellulase by *Trichoderma*, *Biotechnol. Bioeng. Symp.* 6 (1976) 35–53.
- [140] P. Beguin, J.P. Aubert, The biological degradation of cellulose, *FEMS Microbiol. Rev.* 1 (1994) 25–58.
- [141] D. Sternberg,  $\beta$  Glucosidase of *Trichoderma*: its biosynthesis and role in saccharification of cellulose, *Appl. Environ. Microbiol.* 5 (1976) 648–654.
- [142] L. Vallander, K. E. Eriksson, Enzyme recirculation in saccharification of lignocellulosic materials, *Enzyme Microb. Technol.* 9 (1987) 714–720.
- [143] G. Beldman, A.G.J. Voragen, F.M. Rombouts, W. Pilnik, Synergism in cellulose hydrolysis by endoglucanases and exoglucanases purified from *Trichoderma viride*, *Biotechnol. Bioeng.* 31 (1988) 173–178.
- [144] G. Excoffier, B. Toussaint, M.R. Vignon, Saccharification of steam exploded poplar wood, *Biotechnol. Bioeng.* 38 (1991) 1308–1317.
- [145] Z. Xin, Y.B. Qu, P.J. Gao, Acceleration of ethanol production from paper mill waste fiber by supplementation with  $\beta$  glucosidase, *Enzyme Microb. Technol.* 15 (1993) 62–65.
- [146] G. Beldman, F.M. Rombouts, A.G.J. Voragen, W. Pilnik, Application of cellulase and pectinase from fungal origin for the liquefaction and saccharification of biomass, *Enzyme Microb. Technol.* 6 (1984) 503–507.
- [147] T.K. Ghose, V.S. Bisaria, Studies on the mechanism of enzymatic hydrolysis of cellulosic substances, *Biotechnol. Bioeng.* 1 (1979) 131–146.
- [148] L.P. Ramos, C. Breuil, J.N. Saddler, The use of enzyme recycling and the influence of sugar accumulation on cellulose hydrolysis by *Trichoderma cellulases*, *Enzyme Microb. Technol.* 15 (1993) 19–25.
- [149] M. Boyle, N. Barron, A.P. McHale, Simultaneous saccharification and fermentation of straw to ethanol using the thermotolerant yeast strain *Kluyveromyces marxianus* IMB3, *Biotechnol. Lett.* 19 (1997) 49–51.
- [150] B.C. Saha, M.A. Cotta, Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw, *Biotechnol. Progr.* 22 (2006) 449–453.
- [151] B.C. Saha, M.A. Cotta, Enzymatic hydrolysis and fermentation of lime pretreated wheat straw to ethanol, *J. Chem. Technol. Biotechnol.* 82 (2007) 913–919.
- [152] B.C. Saha, A. Biswas, M.A. Cotta, Microwave pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol, *J. Biobased Mater. Bioenergy* 2 (2008) 210–217.
- [153] E. Tomas Pejo, J.M. Oliva, A. Gonzalez, I. Ballesteros, M. Ballesteros, Bioethanol production from wheat straw by the thermotolerant yeast *Kluyveromyces marxianus* CECT 10875 in a simultaneous saccharification and fermentation fed batch process, *Fuel* doi:10.1016/j.fuel.2009.01.014.
- [154] K. Olofsson, A. Rudolf, G. Liden, Designing simultaneous saccharification and fermentation for improved xylose conversion by a recombinant strain of *Saccharomyces cerevisiae*, *J. Biotechnol.* 134 (2008) 112–120.
- [155] S.D. Zhu, Y.X. Wu, Y.F. Zhao, S.Y. Tu, Y.P. Xue, Z.N. Yu, X. Zhang, Fed batch simultaneous saccharification and fermentation of microwave/acid/alkali/H<sub>2</sub>O<sub>2</sub> pretreated rice straw for production of ethanol, *Chem. Eng. Commun.* 193 (2006) 639–648.
- [156] C.F. Huang, T.H. Lin, G.L. Guo, W.S. Hwang, Enhanced ethanol production by fermentation of rice straw hydrolysate without



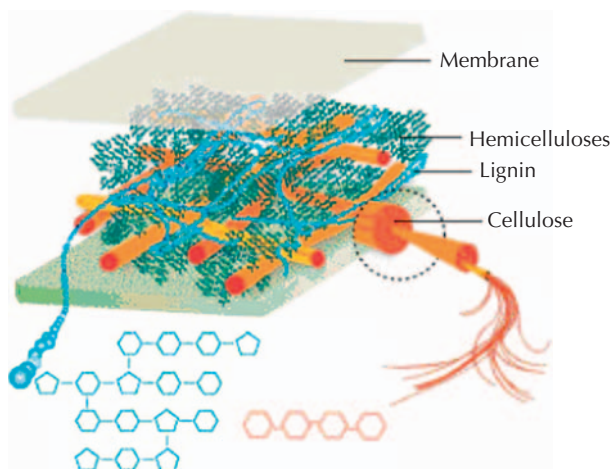
- detoxification using a newly adapted strain of *Pichia stipitis*, *Bioresour. Technol.* 100 (2009) 3914–3920.
- [157] S. Abedinifar, K. Karimia, M. Khanahmadi, M.J. Taherzadeh, Ethanol production by *Mucor indicus* and *Rhizopus oryzae* from rice straw by separate hydrolysis and fermentation, *Biomass Bioenergy* 33 (2009) 828–833.
- [158] B.C. Saha, M.A. Cotta, Enzymatic saccharification and fermentation of alkaline peroxide pretreated rice hulls to ethanol, *Enzyme Microb. Technol.* 41 (2007) 528–532.
- [159] B.C. Saha, M.A. Cotta, Lime pretreatment, enzymatic saccharification and fermentation of rice hulls to ethanol, *Biomass Bioenergy* 32 (2008) 971–977.
- [160] M. Moniruzzaman, L.O. Ingram, Ethanol production from dilute acid hydrolysate of rice hulls using genetically engineered *Escherichia coli*, *Biotechnol. Lett.* 20 (1998) 943–947.
- [161] T.H. Kim, Y.Y. Lee, C. Sunwoo, J.S. Kim, Pretreatment of corn stover by low liquid ammonia recycle percolation process, *Appl. Biochem. Biotechnol.* 133 (2006) 41–57.
- [162] K. Ohgren, O. Bengtsson, M.F. Gorwa Grauslund, M. Galbe, B. Hahn Hagerdal, G. Zacchi, Simultaneous saccharification and co-fermentation of glucose and xylose in steam pretreated corn stover at high fiber content with *Saccharomyces cerevisiae* TMB3400, *J. Biotechnol.* 126 (2006) 488–498.
- [163] K. Ohgren, J. Vehmaanpera, M. Siika Aho, M. Galbe, L. Viikari, G. Zacchi, High temperature enzymatic prehydrolysis prior to simultaneous saccharification and fermentation of steam pretreated corn stover for ethanol production, *Enzyme Microb. Technol.* 40 (2007) 607–613.
- [164] J. Zhao, L.M. Xia, Simultaneous saccharification and fermentation of alkaline pretreated corn stover to ethanol using a recombinant yeast strain, *Fuel Process. Technol.* doi:10.1016/j.fuproc.2009.05.018.
- [165] K. Ohgren, A. Rudolf, M. Galbe, G. Zacchi, Fuel ethanol production from steam pretreated corn stover using SSF at higher dry matter content, *Biomass Bioenergy* 30 (2006) 863–869.
- [166] K. Ohgren, R. Bura, G. Lesnicki, J. Saddler, G. Zacchi, A comparison between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using steam pretreated corn stover, *Process Biochem.* 42 (2007) 834–839.
- [167] L.C. Teixeira, J.C. Linden, H.A. Schroeder, Simultaneous saccharification and cofermentation of peracetic acid pretreated biomass, *Appl. Biochem. Biotechnol.* 84–86 (2000) 111–127.
- [168] K.K. Cheng, J.P. Ge, J.A. Zhang, H.Z. Ling, Y.J. Zhou, M.D. Yang, J.M. Xu, Fermentation of pretreated sugarcane bagasse hemicellulose hydrolysate to ethanol by *Pachysolen tannophilus*, *Biotechnol. Lett.* 29 (2007) 1051–1055.
- [169] W.F. Gauss, S. Suzuki, M. Takagi, Manufacture of alcohol from cellulosic materials using plural ferments, US Patent No. 3990994, 1976.
- [170] H. Golias, G.J. Dumsday, G.A. Stanley, N.B. Pamment, Evaluation of a recombinant *Klebsiella oxytoca* strain for ethanol production from cellulose by simultaneous saccharification and fermentation: comparison with native cellobiose utilising yeast strains and performance in co culture with thermotolerant yeast and *Zymomonas mobilis*, *J. Biotechnol.* 96 (2002) 155–168.
- [171] S.H. Krishna, T.J. Reddy, G.V. Chowdary, Simultaneous saccharification and fermentation of lignocellulosic wastes to ethanol using a thermotolerant yeast, *Bioresour. Technol.* 77 (2001) 193–196.
- [172] J. Hong, Y.H. Wang, H. Kumagai, H. Tamaki, Construction of thermotolerant yeast expressing thermostable cellulase genes, *J. Biotechnol.* 130 (2007) 114–123.
- [173] K.L. Kadam, S.L. Schmidt, Evaluation of *Candida acidothermophilum* in ethanol production from lignocellulosic biomass, *Appl. Microbiol. Biotechnol.* 48 (1997) 709–713.
- [174] Z.W. Wu, Y.Y. Lee, Inhibition of the enzymatic hydrolysis of cellulose by ethanol, *Biotechnol. Lett.* 19 (1997) 977–979.
- [175] Z.W. Wu, Y.Y. Lee, Nonisothermal simultaneous saccharification and fermentation for direct conversion of lignocellulosic biomass to ethanol, *Appl. Biochem. Biotechnol.* 70–72 (1998) 479–492.
- [176] E. Varga, H.B. Klinker, K. Reczey, A.B. Thomsen, High solid simultaneous saccharification and fermentation of wet oxidized corn stover to ethanol, *Biotechnol. Bioeng.* 88 (2004) 567–574.
- [177] Zs. Kadar, Zs. Szengyel, K. Reczey, Simultaneous saccharification and fermentation (SSF) of industrial wastes for the production of ethanol, *Ind. Crops Prod.* 20 (2004) 103–110.
- [178] B. Hahn Hagerdal, H. Jeppsson, L. Olsson, A. Mohagheghi, An interlaboratory comparison of the performance of ethanol producing micro organisms in a xylose rich acid hydrolysate, *Appl. Microbiol. Biotechnol.* 41 (1994) 62–72.
- [179] B.S. Dien, M.A. Cotta, T.W. Jeffries, Bacteria engineered for fuel ethanol production: current status, *Appl. Microbiol. Biotechnol.* 63 (2003) 258–266.
- [180] T.W. Jeffries, Y. S. Jin, Metabolic engineering for improved fermentation of pentoses by yeasts, *Appl. Microbiol. Biotechnol.* 63 (2004) 495–509.
- [181] R.J. Bothast, N.N. Nichols, B.S. Dien, Fermentations with new recombinant organisms, *Biotechnol. Progr.* 15 (1999) 867–875.
- [182] K.A. Gray, L.S. Zhao, M. Emptage, Bioethanol, *Curr. Opin. Chem. Biol.* 10 (2006) 141–146.
- [183] M. Sedlak, N.W.Y. Ho, Production of ethanol from cellulosic biomass hydrolysates using genetically engineered *saccharomyces* yeast capable of cofermenting glucose and xylose, *Appl. Biochem. Biotechnol.* 114 (2004) 403–416.
- [184] R.D. Haan, S.H. Rose, L.R. Lynd, W.H. van Zyl, Hydrolysis and fermentation of amorphous cellulose by recombinant *Saccharomyces cerevisiae*, *Metab. Eng.* 9 (2007) 87–94.
- [185] B.C.H. Chu, H. Lee, Genetic improvement of *Saccharomyces cerevisiae* for xylose fermentation, *Biotechnol. Adv.* 25 (2007) 425–441.
- [186] H.W. Wisselink, M.J. Toirkens, M.D.R.F. Berriel, A.A. Winkler, J.P. van Dijken, J.T. Pronk, A.J.A. van Maris, Engineering of *Saccharomyces cerevisiae* for efficient anaerobic alcoholic fermentation of L arabinose, *Appl. Environ. Microbiol.* 15 (2007) 4881–4891.
- [187] S. Katahira, A. Mizuike, H. Fukuda, A. Kondo, Ethanol fermentation from lignocellulosic hydrolysate by a recombinant xylose and cellobiosaccharide assimilating yeast strain, *Appl. Microbiol. Biotechnol.* 72 (2006) 1136–1143.



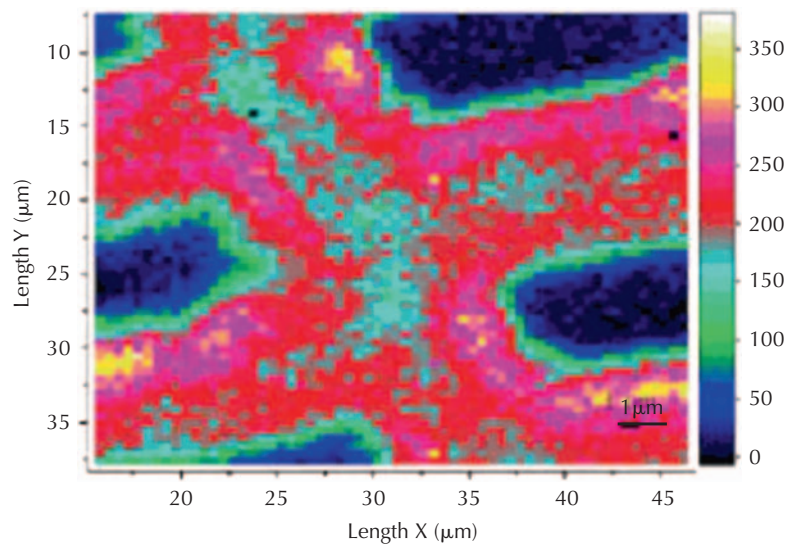
**FIGURE 2.12** Bright field image of partially delignified black spruce wood tissue [30].



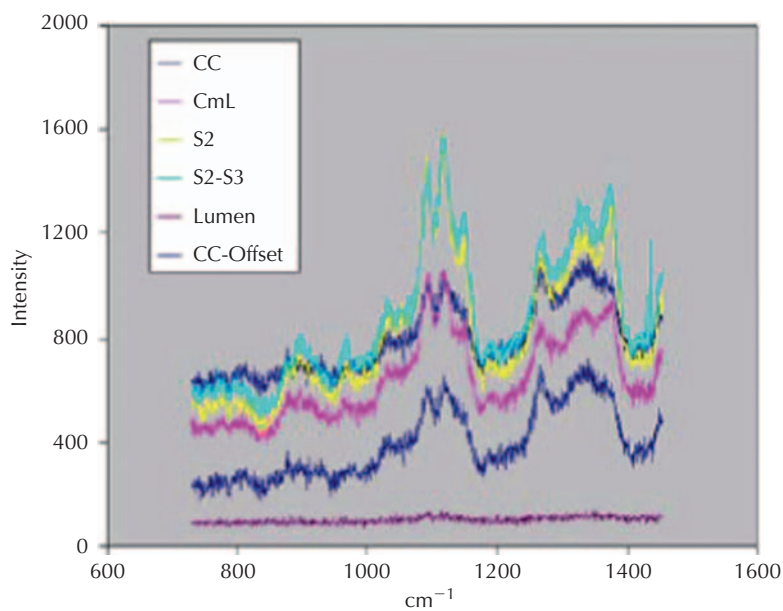
**FIGURE 2.13** Raman images of cellulose spatial orientation and/or distribution in the cell wall [30].



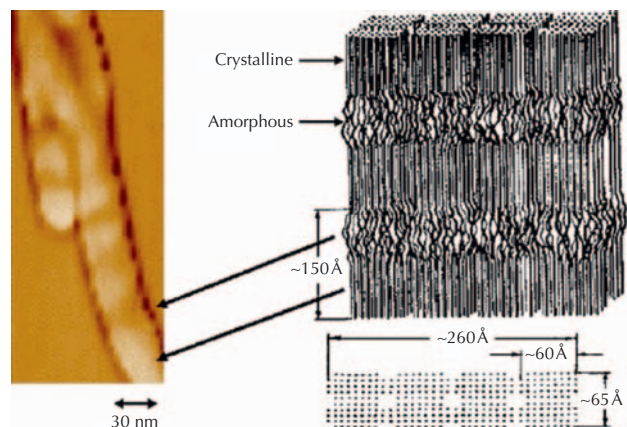
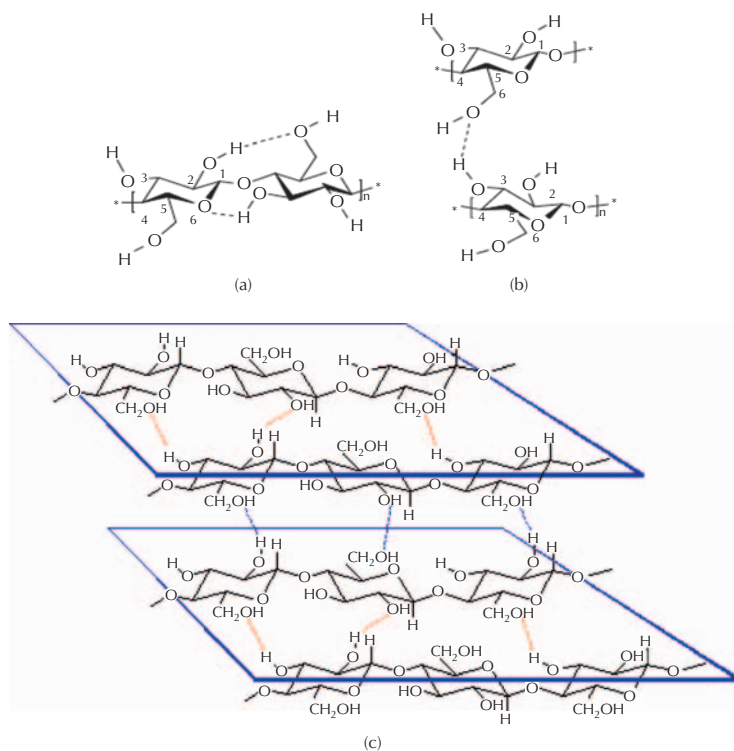
**FIGURE 2.16** The model of cell wall organization [35].



**FIGURE 2.26** Raman images (false color) of cellulose spatial distribution in cell wall [4].

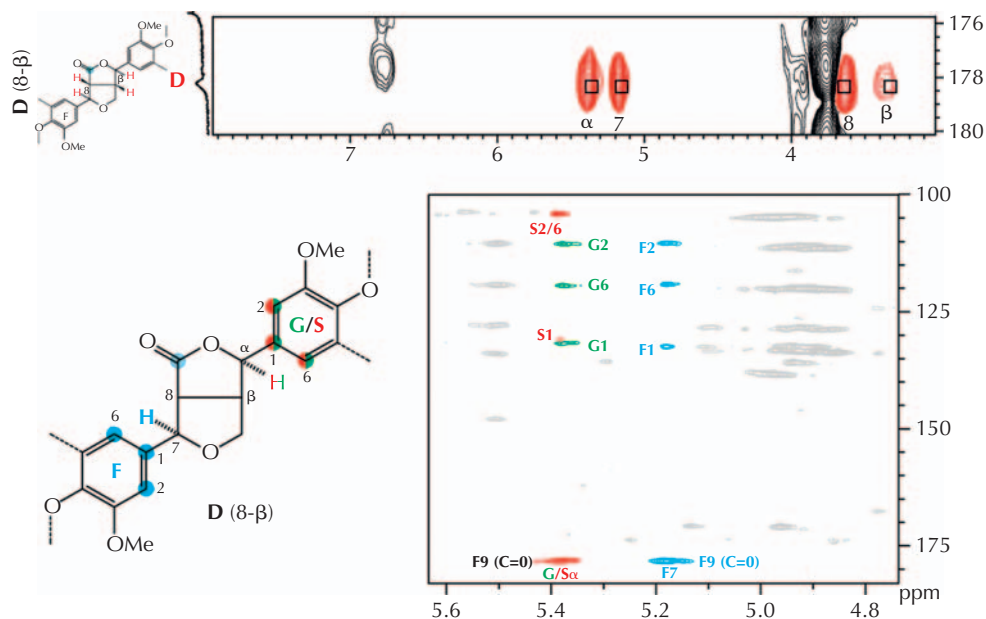


**FIGURE 2.27** Typical spectra in cellulose frequency range (CC offset is same as CC but has been y offset by 400 intensity units for better visualization) [4].



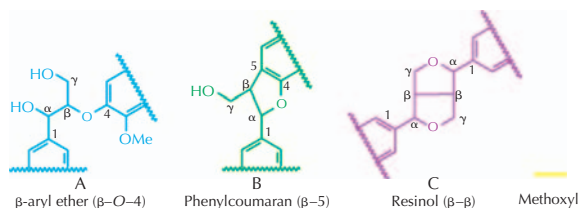
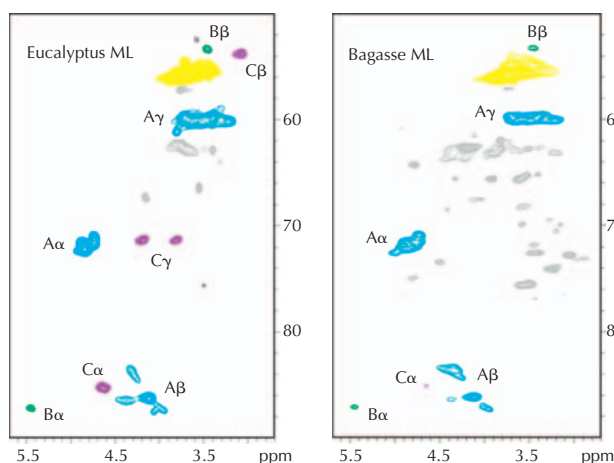
**FIGURE 5.7** Cellulose structure showing (a) the intramolecular hydrogen bonding between C2 OH and C6 OH and C3 OH with endocyclic oxygen, (b) the intermolecular bonding between C3 OH and C6 OH, and (c) the intermolecular bonding between C2 OH and C6 OH and C3 OH [52].

**FIGURE 5.8** Atomic force microscopy phase images support the presence of crystalline and amorphous regions in the direction of the fiber axis [48].

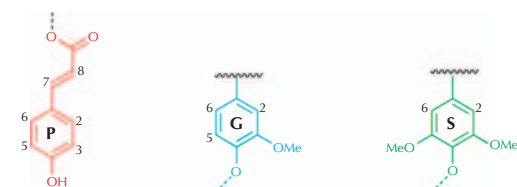
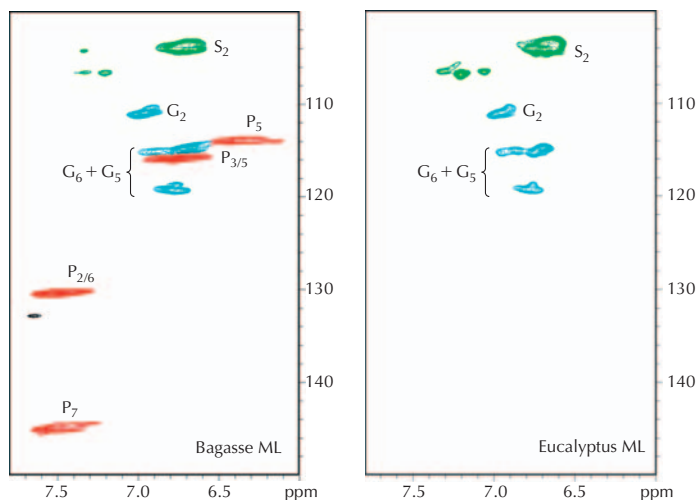


**FIGURE 6.17** HMBC NMR spectrum of a Ryegrass milled lignin that has been 10% <sup>13</sup>C enriched. The required correlations for identifying the 8 β structures of interest, D, are evident in the spectrum. The involvement of guaiacyl and syringyl units in such structures is also verified by the HMBC correlations. Synthesis of valid model compounds is absolutely crucial for the identification of new structures in complex natural polymer such as lignins. (Adapted from Ralph et al. [232]).

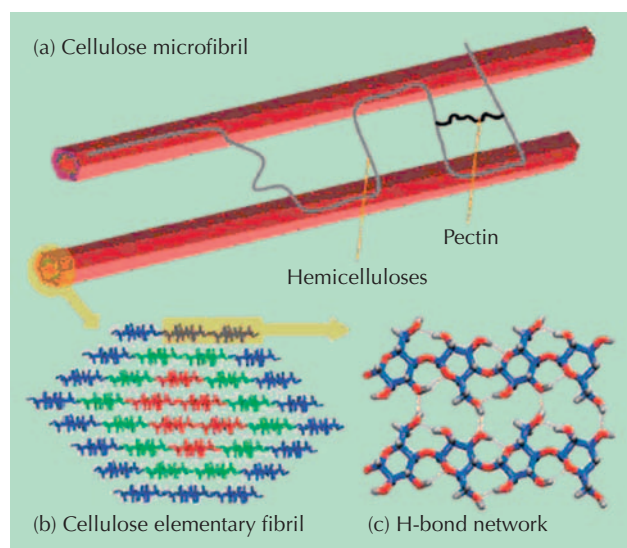




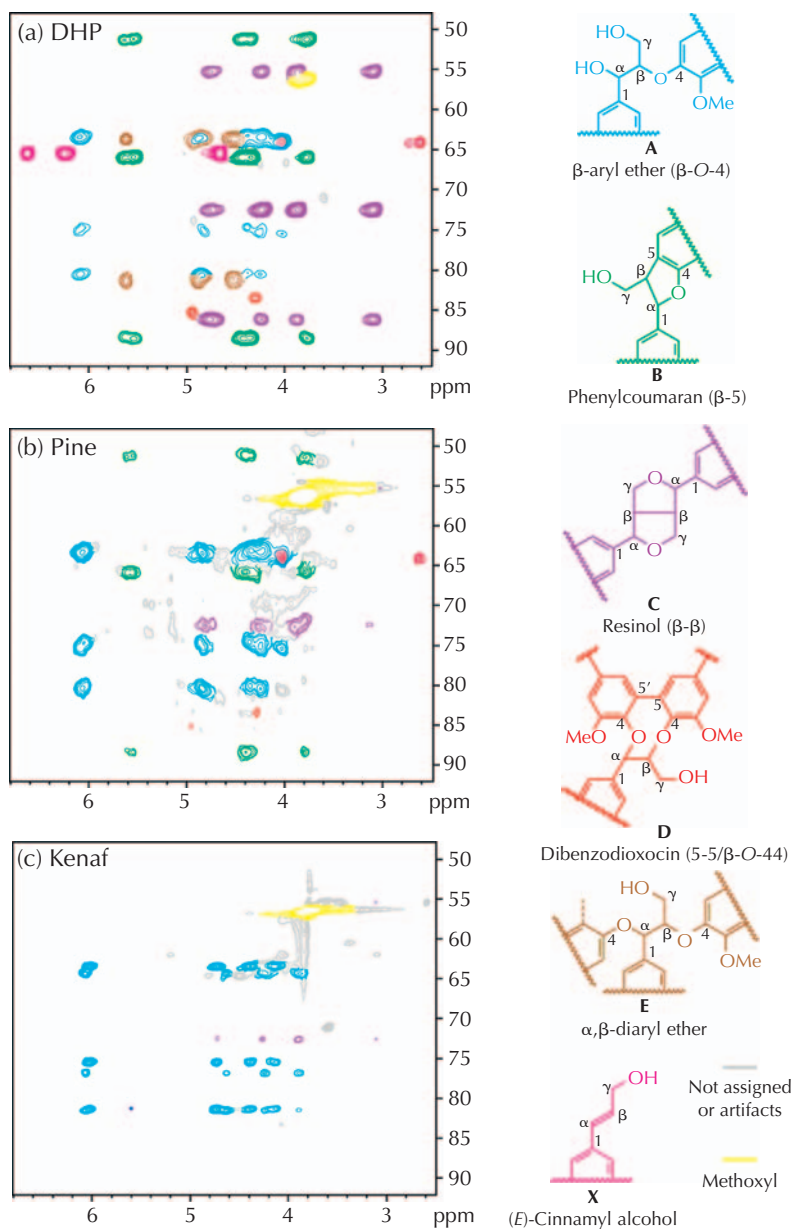
**FIGURE 6.13** Partial HSQC spectra of milled lignins (ML) from Eucalyptus and bagasse showing the sidechain C H correlations from the three main structural units (with  $\beta$  O 4 ,  $\beta$  5 , and  $\beta$   $\beta$  linkages) in lignins.



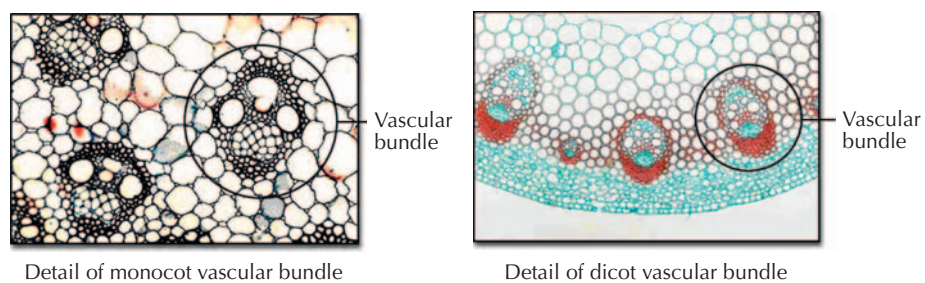
**FIGURE 6.14** Partial HSQC NMR spectra of milled lignins (ML) from Eucalyptus and bagasse showing the C H correlations from aromatic rings of structural units in lignins. The C H correlations from the 2 position of guaiacyl units and the 2/6 positions of syringyl units are well separated, and signals from *p* coumarates on bagasse ML are evident.



**FIGURE 7.5.3** (a) A simplified model showing the interaction of the major polysaccharides in the cell wall. (Lignin is not shown here because its interactions are not well established.) In this system, hemicelluloses are closely associated to the surface of the rigid cellulose crystallite forming the microfibril network. Pectins are cross linked polysaccharides forming a hydrated gel that “glues” the cell wall components together. (b) The 36 chain model of the cellulose elementary fibril. Here, the depiction of the glucan chains is based generally on an X ray structure of cellulose I $\beta$ . It has been proposed that the cellulose elementary fibril may contain three groups of glucan chains: in group C1 (red), there are six true crystalline chains; in group C2 (green), there are 12 subcrystalline chains with a small degree of disorder; and in group C3 (blue), there are 18 surface chains that are subcrystalline with a large degree of disorder. (c) The intra and interchain hydrogen bond network in cellulose I $\beta$  [19].



**FIGURE 6.15** HSQC TOCSY NMR spectra of a synthetic lignin (DHP) and two lignin preparations from Pine and Kenaf cell walls showing the C H correlations from sidechains of structural units with various linkage types in lignins. These C H correlations are aligned along each dimension according to their neighboring protons and protonated carbons (Adapted from Ralph et al. [232]).



**FIGURE 7.5.2** Vascular bundle structures in the monocot and dicot plant, respectively [20].



**A**

- Absorption, 210
  - See also* Sorbency; Sorbents
- Acetylation:
  - cellulose, 155 7
  - straw modification as oil sorbents, 211 12, 215
  - catalysts, 212 13, 214
- Acid hydrolysis:
  - bioethanol production, 270 2
  - concentrated acid hydrolysis, 271 2
  - dilute acid hydrolysis, 272
  - kinetic study, 270 1
  - cellulose, 136
- Acidic extraction, hemicelluloses, 81
- Activated carbons (AC), 241 2
  - production from agricultural byproducts, 244 6
  - chemical activation, 245
  - physical activation, 245
- Acylation:
  - cellulose, 157, 158
  - lignins, 192 3
- Adhesives for wood products, 195 6
- Adsorbents, 4 5
  - See also* Dye removal from aqueous solutions; Heavy metal removal from wastewater
- Adsorption, 210
  - wastewater treatment models, 220 1
  - See also* Sorbency; Sorbents
- Alfalfa:
  - lignin distribution, 27, 31, 32
- Alkali extraction:
  - cellulose, 132 4
  - hemicelluloses, 78 81
  - lignin, 178 80
- Animal feed, 1 2
- Arabino(glucurono)xylans (AGX), 74 5, 76, 90 2, 98, 99, 103 4
- Arabinoxylans (AX), 74, 76, 94, 98, 103
- Ash content, 43
- Atomic force microscopy (AFM), 65 7, 141
- Autohydrolysis, 273 5

**B**

- Bagasse, *See* Sugarcane bagasse
- Bamboo:
  - fiber cell wall, 17 18
  - hemicelluloses, 92
  - lignin distribution, 27
  - vascular bundles, 10
- Banana stem (BS), 18, 25, 27, 33
- Barley straw:
  - extractives, 58, 59 60, 62, 65

- modification for wastewater treatment:
  - dye removal, 242 4
  - heavy metal removal, 233
  - See also* Straw
- Benzylation, hemicelluloses, 114
- Biodegradable plastics, 4
- Bioethanol, *See* Biofuel production
- Biofuel production, 2 4, 153, 267 82
  - acid hydrolysis, 270 2
    - concentrated acid hydrolysis, 271 2
    - dilute acid hydrolysis, 272
    - kinetic study, 270 1
  - enzymatic hydrolysis, 272 82
    - detoxification process, 277, 278
    - enzymatic saccharification, 278 9
    - fermentation strategy, 279 82
    - kinetic study, 277 8
    - pretreatment, 273 6
  - from lignins, 196 7
  - hemicellulose/cellulose interaction, 104 5
  - thermochemical processes, 2 3
    - gasification, 3
    - liquefaction, 3
    - pyrolysis, 2 3
- Biological treatment:
  - cellulose isolation, 136 8
  - dye removal from aqueous solutions, 240
  - pretreatment, 276
  - See also* Enzymatic hydrolysis
- Biomass, 1
  - biochemical processes, 2, 3 4
  - energy production, 2 4
  - See also* Biofuel production
- Brazil, bioethanol production, 268

**C**

- <sup>13</sup>C NMR spectroscopy:
  - cellulose, 144 5
  - extractives, 67 70
  - hemicelluloses, 86 7
  - lignin, 185 6
- Carboxylic acids, 157
- Carboxymethylation:
  - cellulose, 158 60
  - hemicelluloses, 112 13
- Cell wall, *See* Fiber cell wall
- Cellulolytic enzyme lignin (CEL), 177 8
- Cellulose, 31 4, 36, 131 62
  - dissolution of, 146 51
    - inorganic molten salts, 147
    - ionic liquids and analogues, 148 51
    - LiOH/urea, 147 8
    - N* methylmorpholine *N* oxide (NMMO), 147
    - NaOH/urea, 147 8

- distribution, 18 20, 26
- gasification, 259, 261
- hemicellulose interactions, 100 5
  - assembly characteristics of
    - hemicelluloses on cellulose, 105
  - in bioethanol production, 104 5
  - in papermaking process, 103 4
  - in production of celluloses, 101 2
- hydrolysis, 136, 153 4
- isolation, 132 8
  - acid hydrolysis, 136
  - alkali extraction, 132 4
  - alkaline peroxide extraction, 135
  - biological treatment, 136 8
  - delignification, 132 3
  - organic solvent extraction, 135 6
  - steam explosion, 134 5
- liquefaction, 253 4, 255, 256
- modification, 154 62
  - acetylation, 155 7
  - acylation, 157, 158
  - carboxymethylation, 158 60
  - esterification, 157 8
  - tritylation, 161
- occurrence, 131 2
- regeneration of, 151 3
- structure, 138 43
  - cellulose lattice I, 138 41
  - conversion to cellulose II, 142 3
  - structural properties, 143 6
  - supermolecular structure, 138
- Chemical initiation grafting, 223 7
- China:
  - bioethanol production, 268
  - paper production, 2
- Cinnamic acids, 60
- Climate change, 1
- Coconut (COIR), 18, 25, 27, 33
- Complex heteroxylans (CHX), 74, 75, 76, 77
- Confocal laser scanning microscopy (CLSM), 22
- Corn:
  - liquefaction, 256, 258
  - modification for wastewater treatment, 231 2
- Cortex, 9
- Cotton fibers, as sorbents, 216
- p* Coumarates, grass lignins, 192 3
- Cutin, 40 1

**D**

- DEAE cellulose 52 chromatography, 84
- Delignification, 132 3
- Density, sorptive capacity and, 211
- DFRC analytical method, 183 5

Diacylglycerols, 56  
 4 Dimethylaminopyridine (DMAP), 107 8, 213  
 Dimethylsulfoxide (DMSO), 85, 149, 151  
 Dye removal from aqueous solutions, 239 48  
   activated carbon (AC) use, 241 2, 245 6  
   agricultural waste/byproduct use, 242 4, 245 6  
   available technologies, 239 41  
   biological treatments, 240  
   chemical methods, 240  
   physical methods, 240 1  
 Dyes, 239, 240

## E

Electron dispersive X ray analysis (EDXA), 22  
 Energy sources, 2 4, 267  
   *See also* Biofuel production; Biomass  
 Enzymatic hydrolysis, 272 82  
   detoxification process, 277, 278  
   enzymatic saccharification, 278 9  
   fermentation strategy, 279 82  
     separate hydrolysis and fermentation, 281  
     simultaneous saccharification and fermentation, 281 2  
   kinetic study, 277 8  
   pretreatment, 273 6  
     biological pretreatment, 276  
     dilute acid prehydrolysis, 275  
     steam explosion, 273 5  
 Enzymatic mild acidolysis lignin (EMAL), 178  
 Enzyme lignin, 177 8  
 Epidermis, 9  
 Epoxy resin, 258  
 Esterification:  
   cellulose, 157 8  
   hemicelluloses, 106 11  
   modification for wastewater treatment, 222  
 Ethanol, 3, 267  
   hemicellulose fractionation, 83 4  
   production, 3  
     hemicellulose/cellulose interaction, 104 5  
   *See also* Biofuel production  
 Etherification:  
   hemicelluloses, 111 14  
   modification for wastewater treatment, 222  
 Extensin, 94  
 Extractives, 49  
   chemical composition, 52 62  
   lipophilic compounds, 52 60  
   phenolic compounds, 60 2  
   isolation, 50 1  
      Soxhlet extractions, 51  
     supercritical fluid extraction, 51  
   purification, 51  
   spectroscopic characterization, 62 70  
   structural characterization, 52  
   thermal analysis, 70

## F

Fermentation, 279 82  
 Ferulates, grass lignins, 188 92  
 Fiber cell wall, 10 12, 131  
   lignification, 170 4

matrix components, 15  
 polysaccharide distribution, 18 21, 25  
 Formaldehyde, wood adhesives, 195 6  
 Fourier transform infrared (FTIR) spectroscopy:  
   cellulose, 144  
   extractives, 63, 64 6  
   hemicelluloses, 85 6  
 Fractionation, hemicelluloses, 83 5  
 Free fatty acids, 54 6  
 Freundlich isotherm model, 221

## G

Galactoglucomannan, 21  
 Galactomannans, 75 6  
 Gasification, 3, 253  
   hydrothermal gasification, 261 3  
   thermal gasification, 259 61  
 Gas liquid chromatography (GLC),  
   hemicelluloses, 89  
 Global warming, 1  
 Glucomannans, 37, 76  
 Glucurono(arabino)xylans (GAX), 21, 74, 75, 76 7  
 Glucuronoxylan, 21  
 Glucuronoxylans (GX), 74, 98  
 Graft polymerization:  
   hemicelluloses, 115 16  
   modification for wastewater treatment, 223 7  
     chemical initiation grafting, 223 7  
     high energy radiation grafting, 223  
     photografting, 223  
 Granular activated carbon (GAC), 241  
 Grass:  
   anatomical structure, 9 10  
   lignification, 170 4  
   lignins:  
     distribution, 24 9  
     ferulates, 188 92  
     lignin carbohydrate complex (LCC), 193 4  
     *p* coumarates, 192 3  
   ultrastructure, 15 18  
 Greenhouse gas emissions, 270

## H

<sup>1</sup>H spectroscopy:  
   cellulose, 145  
   extractives, 67, 69  
   hemicelluloses, 88  
   lignin, 185  
 Halogenation, 222  
 Heat treatments, hemicellulose extraction, 82 3  
 Heavy metal removal from wastewater, 219 34  
   adsorption models, 220 1  
   biosorption mechanism, 220  
   chemically modified straw based adsorbents, 219 20, 227 33  
   chemical modification methods, 221 7  
   corncoobs, 231 2  
   rice straw/husks/hull, 232 3  
   soybean straw/hull, 232 3  
   sugarcane bagasse, 227 30  
   wheat straw/bran, 231

Hemicelluloses, 34 7, 73 119  
   applications, 117 18  
   biological activity, 100  
   cellulose interactions, 100 5  
     assembly characteristics of  
       hemicelluloses on cellulose, 105  
     in bioethanol production, 104 5  
     in papermaking process, 103 4  
     in production of celluloses, 101 2  
   classification, 74 6  
   distribution, 19, 20 1, 27  
   gasification, 259, 261  
   isolation, 77 85  
     chemical reagents, 77 81  
     combined chemical and mechanical treatment, 81 2  
     fractionation and purification, 83 5  
     heat treatments, 82 3  
     membrane, 83  
   liquefaction, 253  
   modification, 105 17  
     esterification, 106 11  
     etherification, 111 14  
     graft polymerization, 115 16  
     oxidation, 116 17  
     potential modification, 105 6  
   *M<sub>w</sub>*, 96 7  
   occurrence, 73 4, 76  
   rheological behavior, 97 8  
   solubility, 96  
   straw hemicelluloses, 76 7  
   structural analysis, 85 9  
     chromatography, 89  
     mass spectrometry, 89  
     nuclear magnetic resonance spectroscopy, 86 8  
     optical spectroscopy, 85 6  
   structure, 89 96  
   surface tension, 98 9  
   thermal behavior, 99  
 Heteroxylans (HX), 74  
 High energy radiation grafting, 223  
 High performance liquid chromatography (HPLC), hemicelluloses, 89  
 High boiling solvent (HBS) lignin, 181 2  
 Homoxylans (X), 74  
 Hot water extraction, hemicelluloses, 82  
 Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>):  
   cellulose isolation, 135  
   hemicellulose isolation, 79 81  
   lignin isolation, 180  
 Hydrolysis, cellulose, 153 4  
 Hydrothermal gasification, 261 3  
 Hydrothermal liquefaction, 253 6

## I

Industrial wastewater treatment, *See* Wastewater treatment  
 Insoluble straw xanthate (ISX), 231  
 Ionic liquids (IL), 148 51, 152 3  
 Isolation:  
   cellulose, 132 8  
   extractives, 50 1  
   hemicelluloses, 77 85  
   lignins, 174 83

**K**

Ketosterols, 56

**L**

Langmuir isotherm model, 221

Lignification, 170 4

lignin deposition, 171 3

monolignol biosynthesis, 170 1

monolignol polymerization, 173 4

Lignin, 21 2, 37 9, 169 98

acylation, 192 3

analytical methods, 183 7

DFRC method, 183 5

NMR spectroscopy, 185 7

thioacidolysis, 183

applications, 194 7

biofuel, 196 7

wood adhesives, 195 6

definition, 169 70

deposition, 171 3

distribution, 19, 21 9, 30, 31

grass, 24 9

wood, 23 4

gasification, 259

isolation, 174 83

alkali lignins, 178 80

enzyme lignin, 177 8

high boiling solvent lignin, 181 2

hydrogen peroxide lignin, 180

milled wood lignin (MWL), 175 7

organosolv lignin, 180 2

liquefaction, 253 4, 255

purification, 182 3

structural characteristics, 187 94

ferulates in, 188 92

lignin carbohydrate complex (LCC),  
193 4

*p* coumarates, 192 3

topochemistry, 171 3

*See also* Lignification

Lignin carbohydrate complex (LCC), 193 4

Lignocellulosic biomass recalcitrance,  
268 70

Lignocellulosic ethanol production, 3

LiOH/urea, 147 8

Lipophilic compounds, 52 60

Liquefaction, 3, 253

hydrothermal liquefaction, 253 6

solvolytic liquefaction, 256 8

Livestock feed, 1 2

**M**

Mannans, 75 6

Mass spectrometry, hemicelluloses, 89

Matrix assisted laser desorption/ionization time  
of flight (MALDI TOF) mass  
spectroscopy, hemicelluloses, 89

Matrix components, 15

Membrane extraction, hemicelluloses, 83

Methylation, hemicelluloses, 114

Microcrystalline cellulose (MCC), 138

Microfibril angle (MFA), 12 15

Microfibrils, 12 14, 131

Microwave assisted extraction, hemicelluloses,  
82 3

Milled wood lignin (MWL), 175 7

Minerals, 43

Molten salt hydrates, 147, 148

Monolignols, 170 1, 173 4

biosynthesis, 170 1

polymerization, 173 4

**N**

*N* bromosuccinimide (NBS), 213, 215

*N* methylmorpholine *N* oxide (NMMO), 147,  
151 2, 153 4

NaOH/urea, 147 8

Nonisothermal simultaneous saccharification  
and fermentation process (NSSF), 282

Nuclear magnetic resonance (NMR)

spectroscopy:

cellulose, 144 6

extractives, 67 70

hemicelluloses, 86 8

lignin, 185 7

**O**

Oil palm frond (OPF), 18, 25, 27 9, 33

Oil spill:

control methods, 209

hazard, 209

sorbents, 4 5

agro based sorbent application, 213 16

chemical modification of cereal straws,  
211 13

classification, 209 10

Optical spectroscopy, hemicelluloses, 85 6

Organosolv:

cellulose extraction, 135

hemicellulose extraction, 81

lignin extraction, 180 2

Oxidation:

hemicelluloses, 116 17

modification for wastewater treatment, 222 3

**P**

Paper production, 2

cellulose isolation, 132, 133

hemicellulose/cellulose interaction, 103 4

Pectic substances, 39

Phenolic compounds, 60 2

Phloem, 9

Photografting, 223

Pineapple leaf (PALF), 18, 25, 27, 33

Pollution, *See* Heavy metal pollution; Oil spill

Polysaccharide distribution, 18 21, 25

Polyurethane foam (PUF), 258

Porosity, sorptive capacity and, 211

Powdered activated carbon (PAC), 241

Proteins, 40

Purification:

extractives, 51

hemicelluloses, 83 5

lignins, 182 3

Pyridine catalyzed acetylation, 213

Pyrolysis, 2 3

**R**

Raman microscopy, 14 15, 16, 22 3

Raman spectroscopy, 65, 68

Rapeseed methyl ether (RME) biodiesel, 2

Reed fiber, 16, 22

lignin distribution, 27

Retention, sorptive capacity and, 211

Rice straw:

extractives, 56 8, 62, 64

hemicelluloses, 91

liquefaction, 256

modification for wastewater treatment:

dye removal, 242

heavy metal removal, 230 1

*See also* Straw

Rye straw extractives, 58, 59 60, 62, 65

**S**

Saccharification, 278 9, 281 2

Sclerenchyma, 10

Selectivity, sorptive capacity and, 211

Simultaneous saccharification and

co fermentation (SSCF), 282

Simultaneous saccharification and fermentation  
(SSF), 281 2

Sodium carboxymethylcellulose (CMC),  
158 60

Solar energy, 268

Solid phase extraction (SPE), 51

Solvolytic liquefaction, 256 8

Sorbency, 210

influences on sorptive capacity, 210 11

density, 211

porosity, 211

retention, 211

selectivity, 211

Sorbents, 4 5

agro based sorbent application, 213 16

chemical modification of cereal straws,  
211 12

catalysts in chemical modification,

212 13, 214

classification of oil sorbents, 209 10

Soxhlet extractions, 51

Spruce tracheid ultrastructure, 19, 22,  
27, 28

Steam explosion, 104, 273 5

cellulose isolation, 134 5

hemicellulose isolation, 82

Sterols, 56

Steryl esters, 56

Straw, 1, 5

anatomical structure, 9 10

chemical composition, 29 43

*See also* Extractives

lignin distribution, 21 9, 30

modification as sorbents:

dye removal from aqueous solutions,  
239 48

heavy metal removal from industrial  
wastewater, 219 34

oil spill cleanup, 209 16

polysaccharide distribution, 18 21, 25

production, 1

ultrastructure, 10 18

*See also* Cellulose; Hemicelluloses;

Lignin

Suberin, 41 2

Sugarcane bagasse (SCB), 227  
     hemicelluloses, 90 1  
     liquefaction, 257  
     modification for wastewater treatment,  
         227 30  
     ultrastructure, 16 17, 23  
 Sunflower stalk liquefaction, 254, 256  
 Supercritical antisolvent precipitation, 84 5  
 Supercritical fluid extraction (SFE), 51  
 Swelled enzyme lignin (SEL), 178  
 Switchgrass gasification, 261  
 Syngas, 260 1

## T

Thermal analysis, extractives, 70  
 Thermal gasification, 259 61  
 Thioacidolysis, lignin, 183  
 Triglycerides, 56  
 Tritylation, cellulose, 161

## U

Ultrasound assisted extraction, hemicelluloses,  
     81 2  
 United States, bioethanol production,  
     267 8

## V

Vascular bundles, 9 10  
 Verbascum stalk liquefaction, 254

## W

Wastewater treatment:  
     chemically modified straw, 227 33  
         corncobs, 231 2  
         modification methods, 221 7  
         rice straw/husks/hulls, 230 1  
         soybean straw/hull, 232 3  
         sugarcane bagasse, 227 30  
         wheat straw/bran, 231  
     dye removal, 239 48  
         activated carbon (AC) use, 241 2,  
             245 6  
         agricultural waste/byproduct use, 242 4,  
             245 6  
         available technologies, 239 41  
         heavy metal removal, 219 34  
         adsorption models, 220 1  
         mechanism of metal biosorption, 220  
 Waxes, 42 3, 49, 56  
 Wheat straw:  
     chemical composition, 34

extractives, 52 6, 58 63, 64 5, 67 70  
 hemicelluloses, 90  
 lignin distribution, 24 7, 31  
 liquefaction, 257, 258  
 ultrastructure, 16, 19, 20  
 use for dye adsorption, 242  
*See also* Straw

Wide angle X ray diffraction (WAXD) curves,  
     cellulose, 140

### Wood:

    anatomical structure, 9  
     lignin distribution, 21, 23 4, 30  
     polysaccharide distribution, 18 21  
     ultrastructure, 10 15

Wood adhesives, 195 6

Wood based materials, 4

## X

X ray diffraction (XRD), cellulose, 138 40,  
     142 3

Xylans, 35 7, 74 7, 118

*See also* Hemicelluloses

Xylem, 9

Xylitol, 118

Xyloglucans (XG), 37, 76, 103